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Ph. D. THESIS
(Summary of Ph. D. thesis)

SUPERABSORBENT HYDROGELS OBTAINED BY RADIATION CROSSLINKING

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CONTENT
(*Corresponding to the Ph.D. thesis*)

INTRODUCTION

Hydrogels are a group of polymeric materials, with three-dimensional hydrophilic structure and attractive physico-chemical properties for a number of applications in various fields. The growing demand for the development of new hydrogels is mainly due to their wide range of applications, namely: as hygiene and personal care products, pharmaceuticals and cosmetics, in food and agriculture and, more recently, in tissue engineering or regenerative medicine.

The radiation cross-linking method for the production of hydrogels is considered the best choice because it offers a number of advantages to both the manufacturer and the final product, as follows:

- ✓ The technology is environmentally friendly with no additional costs in terms of waste management;
- ✓ The crosslinking reaction can also be carried out without the addition of catalysts or chemical initiators;
- ✓ The final product is obtained in only one technological stage, being possible, both obtaining the hydrogel and its final sterilization;
- ✓ Synthesis of polymeric hydrogels by crosslinking with electron beams (e-beam) also offers the advantage of using increased dose rates compared to ^{60}Co irradiation, so the final product is obtained in a short time (*i.e* for applying a 25 kGy dose, the legal dose of sterilization, it takes just 5 minutes at a dose rate of 5 kGy/min; The radio-induced oxidation processes that typically occur when a polymeric mixture is exposed to ionizing radiation for a long time are significantly reduced.

Given the benefits of hydrogel materials and the growing interest in using these materials in tissue engineering and regenerative medicine, the present work aims to develop superabsorbent hydrogels made from natural and synthetic polymers prepared exclusively by treatment with ionizing radiation (gamma and e-beam). The main materials selected for the production of these hydrogels were collagen obtained from calf skin, poly (vinylpyrrolidone) (PVP) and poly (ethylene oxide) (PEO).

From the literature survey, no collagen-PVP or collagen/PVP/PEO hydrogels were obtained by γ-radiation processing or e-beam in the absence of oxygen, inert atmosphere or in the presence of air.

In order to optimize a hydrogel for a final product, such as the integration of biomacromolecules or drugs in it, a good understanding of the relationship between the absorbed dose and the final properties of the hydrogel is required. Thus for a target application, the hydrogels were characterized from structural and compositional point of view, and the network parameters were correlated with the radiation absorbed dose. Also, the cross-linking and degradation yields, the degree of swelling, the stability of hydrogels in environments with different pH, the rheological behavior, the kinetics of water diffusion and the chemical structure were investigated according to the absorbed dose.

The thesis is divided into two parts:

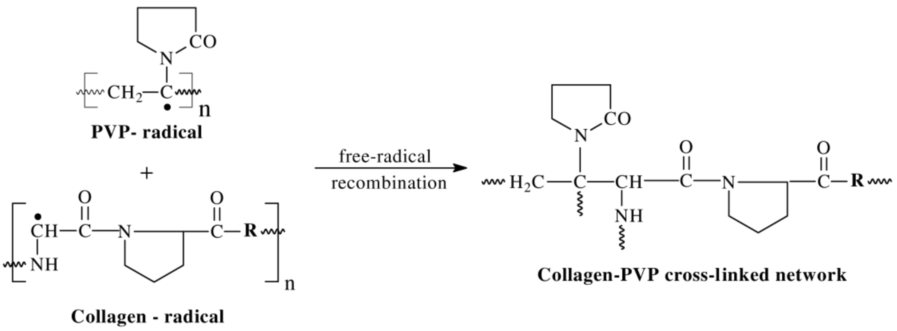
- ✓ Part I includes the literature study and is structured in 3 chapters;
- ✓ Part II includes the original results, general conclusions and bibliographic references and is structured in chapters 5 - 9.

CHAPTER 5. GAMMA RADIATION CROSSLINKING OF COLLAGEN-PVP SUPERABSORBENT HYDROGELS

Based on the reaction mechanisms existing in the literature for collagen γ-radiation cross-linking [2-4], we proposed a crosslinking mechanism for the collagen-PVP system according to Scheme 5.1. The reaction takes place through the specific recombination of PVP free radicals with a collagen radical, with the formation of a covalent bond between the PVP macroradicals and collagen. These links stabilize the triple-helix structure of collagen that is most susceptible to degradation during the radiation cross-linking process.

5.1. Sol-gel analysis

The evaluation of the radiation cross-linking process was performed using the sol-gel analysis and determining the parameters: G(X) and G(S) - cross-linking and degradation yields; Dg - gelation dose [6]. The gel fraction is raised even at low doses (at 5 kGy exceeds 65%) (figure 5.1a). Dg, the ratio p_0/q_0 (degradation vs. crosslinking) was calculated, using freely available software, which has implemented the Charlesby-Pinner and Charlesby-Rosiak equations [7]. In the case of the thesis study, the maximum value of G(X) was 3.65×10^{-8} mol J^{-1} with a decreasing tendency with increasing of irradiation dose. G(S) was more than one order smaller than G (X), also having a decreasing tendency with dose. The crosslinking density (Ve) increases with the absorbed dose and a denser



Scheme 5.1. Reaction mechanism for γ-radiation crosslinking of collagen-PVP hydrogel [5]

hydrogel structure appears, which leaves less possibilities for the formation of new covalent bonds and degradation processes through cleavage reactions of macromolecular chains.

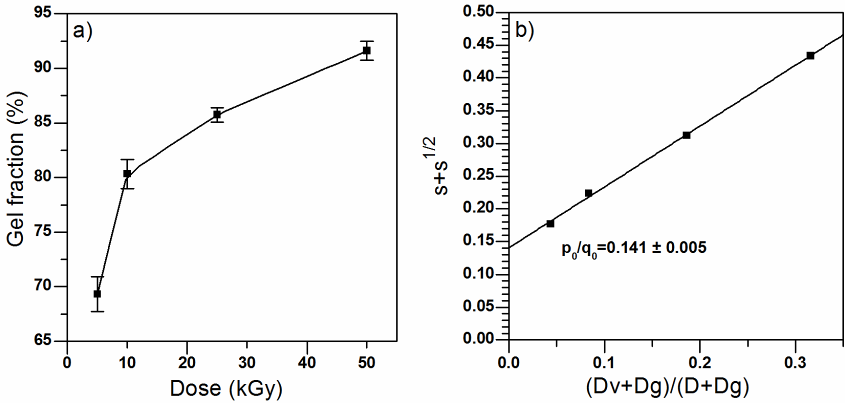


Figure 5.1. a) The gel fraction; b) Sol-gel analysis according to the Charlesby-Rosiak equation [1]

5.2. The swelling degree, the diffusion of water and the mechanism of swelling process

The absorption capacity, the network structure and the effective crosslinking density of the collagen-PVP hydrogels were investigated by determining the properties of swelling degree in deionised water (figure 5.2). From the figure it is observed that the equilibrium is reached after 12 hours, which means that a stable hydrogel with a dense structure has formed. The degree of swelling decreases with increasing of dose in the crosslinking process and shows that the Ve increases with dose increasing. The study on the diffusion and the mechanism of the swelling process was conducted to initiate and support future studies aimed to integrate biomacromolecules or drugs into the matrix of collagen and PVP-based hydrogels.

For the characterization of the swelling kinetics process, the parameters k, n and the diffusion coefficients (D) were determined. For a non-Fickian diffusion mechanism, the crosslinking density is increased, which means a small amount of water is included and which obviously leads to a decrease in the diffusion rate [8, 9]. The values of (k), (n) and (D) parameters for the studied hydrogels, in relation to the irradiation dose, are presented in table 5.1. The values of (n) are in the range 0.58-0.68 and decrease with increasing of irradiation dose, which indicates that the type of water diffusion in collagen-PVP hydrogels is non-Fickian. The non-Fickian transport type is specific for the cross-linked hydrogels [8, 9], so that its (n) values may also be related to the mechanism of transport of drugs or active substances.

Table 5.1. Parameters $\boldsymbol{k}$, $\boldsymbol{n}$ și $(\boldsymbol{D})$

Dose (kGy)	k	n	R^2	D [cm^2 s^{-1}]
5	-2,56	0,68	0,99	0,08
10	-2,28	0,66	0,99	0,11
25	-2,31	0,58	0,95	0,09
50	-2,38	0,59	0.94	0,07

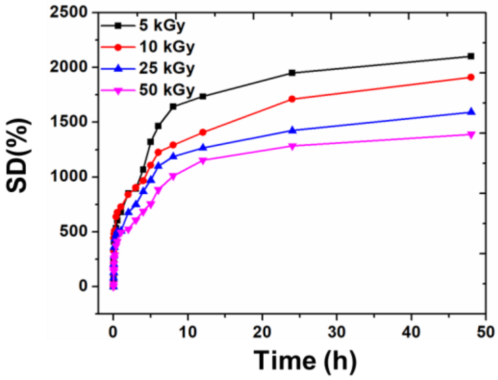


Figure. 5.2. Swelling degree of the hydrogel vs. immersion time in water

5.3. Rheological analysis

The collagen-PVP hydrogels showed an elastic modulus (G') in good approximation constant in the range (0.1–10) rad/s. During this interval, a predominantly elastic behavior was observed for all hydrogels obtained by irradiation, which suggests the formation of a permanent network. From figure 5.3 it is observed that G' increases up to 25 kGy (it reaches 100 Pa), and at the dose of 50 kGy, it decreases, which means that at higher radiation doses in the hydrogel structure can occur bonds scission, visible in decreasing the cross-linking density with increasing the dose.

5.4. ATR-FTIR spectroscopy

Structural changes that occurred after γ-irradiation in collagen-PVP hydrogels were monitored by ATR-FTIR spectroscopy. In the range (3800-2600) cm^{-1} we observed an increase of the intensity of the amide A and B bands characteristic of collagen and the stretching vibrations of the functional groups as OH, CH and CH_2 (figure 5.4). The most important absorption band shifts to smaller wave number values were found for the irradiated sample at 50 kGy (3320 cm^{-1} to 3308 cm^{-1}); the position of the amide B (3075 cm^{-1}) remained unchanged. The formation of H bonds or other interactions between the chemical groups of two different polymers usually causes the change of the peak positions of the participating groups. The interactions that take place by the formation of H bonds, move the stretching vibrations of the OH type groups, towards smaller wave numbers, accompanied by the increase of the intensity and the widening of the characteristic peak [10]. With the increase of the absorbed dose from 5 kGy to 50 kGy in the range (2850-2950) cm^{-1} the increasing of bands intensity was observed. From FTIR data was verified whether the triple-helix structure of collagen was damaged by irradiation by calculating the ratio of peak intensity of amide III (1240 cm^{-1}) to peak intensity at 1450 cm^{-1} [11]. It was observed that following the irradiation of the collagen-PVP mixture, the value of this ratio decreases with increasing dose, from 0.96 to 0.79 for the (5-50) kGy range, which suggests that the triple-helix structure of collagen has suffered a deterioration for doses greater than 25 kGy, and for doses less than 10 kGy, crosslinking reactions are characteristic.

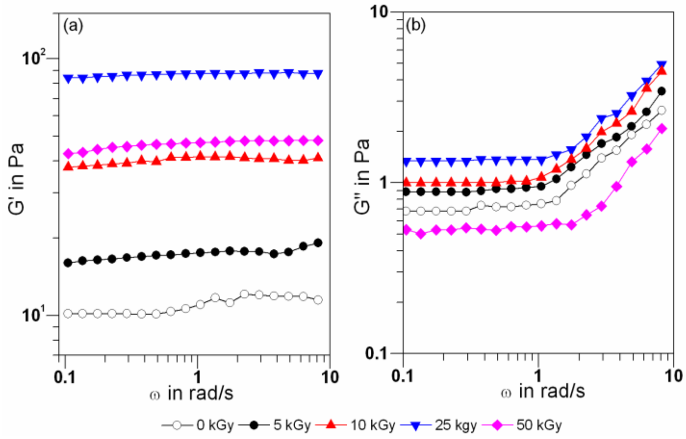


Figure 5.2. (a) Variation of the G' (a) și G'' (b) moduli vs. angular velocity

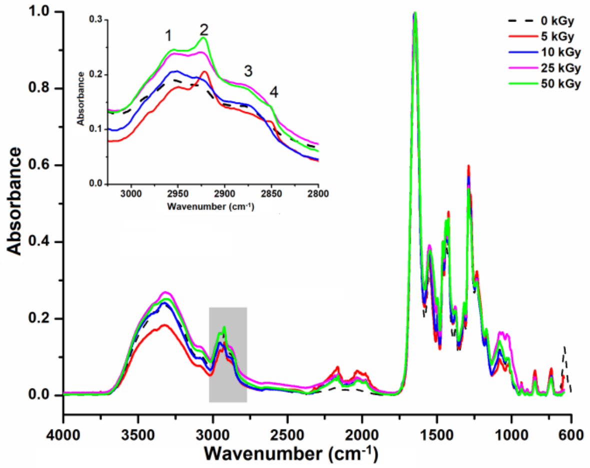


Figure 5.3. FT-IR spectra for collagen–PVP hydrogels

5.5. Hydrogel network structure

The experimental values for the molecular mass between two crosslinking points (M_C), the crosslinking density (Ve), the mesh size (ξ) and și $\nu_{2,r}$, $\nu_{2,s}$, $\rho_{polimer}$ are presented in table 5.2. The Ve is a parameter which generally describes the characteristics of the gel and which increased with the absorbed dose.

Table 5.2. The network parameters obtained for the collagen-PVP hydrogel

Dose (kGy)	ρ (g cm^{-3})	$\nu_{2,r}$	$\nu_{2,s}$	$V_e \times 10^{-5}$ (mol · cm^{-3})	$M_C \times 10^4$ (g · mol^{-1})	ξ (nm)
5	1,26	0,11	0,04	1,24	8,09	134
10	1,12	0,12	0,04	1,95	5,12	99
25	1,19	0,12	0,05	2,00	5,00	95
50	1,22	0,13	0,06	2,95	3,39	75

The parameters M_C and ξ were found in the range 3.39–8.08×10^4 g·mol^{-1}, and 75–134 nm; both decrease with the absorbed dose. The decrease of the mesh size according to the radiation dose is due to the reduction of the available space between the macromolecular chains of the hydrogel, being an important factor that ensures the mechanical resistance, degradability and diffusivity of the water molecules in the hydrogel network. The mesh sizes obtained in this study are comparable to the diameter of the protein molecules [12]. With this in mind, it is possible to diffuse biomacromolecules into and from the collagen-PVP hydrogel network, as well as drugs with lower molecular weight and other pharmaceutical compounds.

CHAPTER 6. E-BEAM RADIATION CROSSLINKING OF COLLAGEN-PVP SUPERABSORBENT HYDROGELS – OBTAINED IN THE ABSENCE OF AIR

In this study hydrogels with superabsorbent properties based on collagen and PVP were obtained by e-beam processing in the absence of air, with and without the addition of chemical cross-linking agents soluble in aqueous media. The aim of the study was to obtain a hydrogel that would be the basis of a dressing for the rapid wounds healing.

6.1. Collagen/PVP hydrogels prepared without the addition of crosslinking agents

• The degree of swelling

From figure 6.1 it is observed that the degree of swelling of hydrogels 1_C-PVP and 2_C-PVP is maxim at the irradiation dose of 5 kGy and minimum at the dose of 40 kGy. For the hydrogel 3_C-PVP, the maximum is at 40 kGy and the minimum at 5 kGy. In conclusion for hydrogels 1_C-PVP and 2_C-PVP, the degree of swelling decreases with increasing dose, and for hydrogel 3_C-PVP (highest concentration of PVP), the degree of swelling increases with increasing dose.

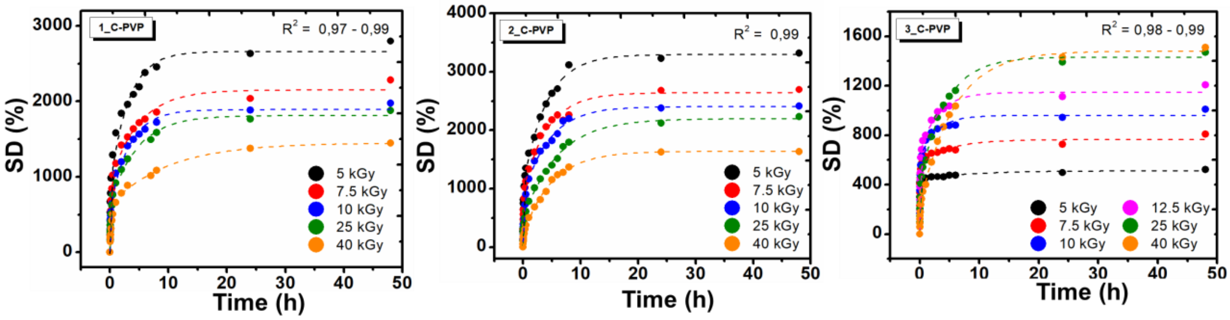


Figure 6.1. The swelling degree as a function of the immersion time and absorbed dose (in deionised water)

•Rheological analysis

Elastic (G') and viscous (G") modules highlight the stability and viscoelastic character of hydrogels in the linear-viscoelastic region and are influenced by the irradiation dose. The linear response of the G' modulus according to the angular velocity (ω) up to a certain limit of the applied force shows that the newly formed macromolecular network opposes the external force and maintains equilibrium. On the contrary, when the force increases to a certain level, the chemical bonds in the hydrogel network, either break or begin to deform, the hydrogel loses its elasticity and becomes viscous. When the viscosity prevails, the G' modulus should be larger than the G" modulus [13]. The values of G' > G", shows that the hydrogel is elastic, as expected for elastic solids [14]. Moreover, it shows that the hydrogels obtained by e-beam crosslinking have a permanent network due to the new bonds formed by cross-linking [15-18]. The increase or decrease of G' with the absorbed dose shows the predominance of crosslinking or degradation of the hydrogel macromolecular network [19].

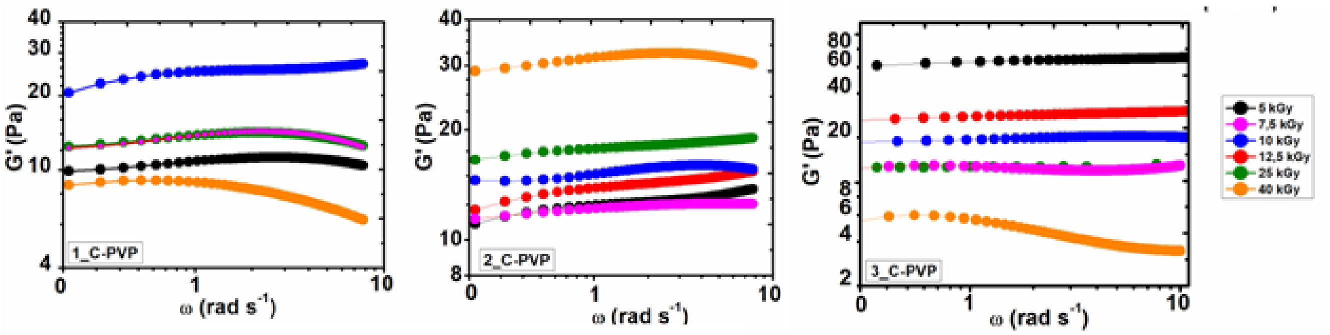


Figure 6.2. Variation of G' modulus depending on the angular velocity (ω) and the absorbed dose

• Crosslinking density (Ve)

Crosslinking density (Ve), is a parameter that describes the number of crosslinking points formed between different polymeric chains (intermolecular crosslinking) or inside the same polymer chain (intra-molecular crosslinking) [20]. In the case of radiation cross-linking, Ve depends on the absorbed dose and is expressed as a function of the molecular mass between two successive cross-linking points (M_C) and the mesh size of the hydrogel network (ξ), values presented in columns (3-5) of table 6.1. The size of parameters M_C and ξ determines the structure of the hydrogel and highlights a lower or higher crosslinking density of the hydrogel. Each hydrogel composition revealed an independent behavior depending on the dose absorbed. For all 3 hydrogels the optimal irradiation dose for crosslinking is in the range (10–25) kGy, above this range, the values of M_C and ξ suggest the existence of degradation processes. The definitions of M_C, Ve and ξ, suggest correlations between the degree of crosslinking and the structure of the hydrogel formed by irradiation, in the sense that a small G' means large M_C and ξ and low Ve.

Table 6.1. Experimental values for G′, G′′, M_C, Ve and ξ

3_C-PVP					
Dose (kGy)	$\bar{G}'$ (Pa)	$\bar{G}''$ (Pa)	M_C (kg mol^{-1})	V_e (mol m^{-3})	ξ (nm)
5	66,5	13,2	1979	0,524	157
7,5	28,4	2,0	4127	0,248	274
10	19,7	0,9	5589	0,182	337
12,5	19,5	1,2	5054	0,200	345
25	12,6	0,4	7557	0,134	417
40	4,8	0,7	21737	0,046	626

6.2. Collagen/PVP hydrogels prepared with crosslinking agents

The addition of acrylic acid (AA) and N, N-methyl-bis-acrylamide (NMBA) was intended to increase the yield of the cross-linking reaction.

- **Sol-gel analysis**

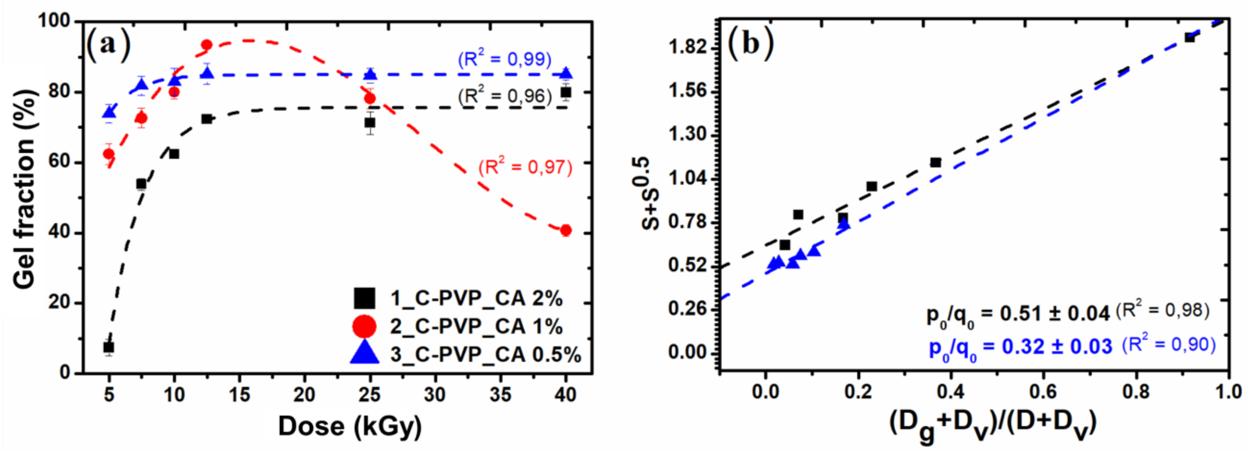


Figure 6.3. (a) Gel fraction; (b) Sol-gel analysis corresponding to the Charlesby-Rosiak equation

For polymeric mixtures prepared with (0.3 - 0.5)% collagen and (5 - 7)% PVP, the gel fraction had values greater than 60% (5 kGy). In the case of 2_C-PVP_NMBA 1% system, the gel fraction decreased drastically at doses greater than 12.5 kGy. For the system in which the concentration of PVP was increased to 7%, and the concentrations of AA and NMBA were reduced to 5.2%, respectively 0.5%, a significant increase of the gel fraction in relation to the absorbed dose was observed, over 75% at 5 kGy. For polymeric mixtures having an insoluble fraction and a ratio p_0/q_0 usually less than 2, indicates that cross-linking processes have predominated [21]. For C-PVP hydrogels prepared with (1-2)% of cross-linking agent, although the values of the ratio p_0 /q_0 = 0.79, respectively 0.51 and the values determined for the radio-chemical yields, G(X) and G(S), (table 6.2), showed rather the presence of degradation processes. From the evaluation of the obtained data, it is evident that the addition of an increased concentration of crosslinking agent this influenced the crosslinking process of the C-PVP hydrogels and the values of the p_0 /q_0 ratios were found considerably higher compared to the values determined for the hydrogels prepared without NMBA. Concentration of the cross-linking agent, the concentration of the polymers must be carefully controlled so that the cross-linking reaction occurs under optimal conditions. For the 3_C-PVP_NMBA 0.5% hydrogel the highest gel fraction was obtained, over 87.2% at the irradiation dose of 7.5 kGy, and at 40 kGy slightly decreased to 82%.

- **Crosslinking and degradation yields**

The highest value of parameter G(X) was determined for 1_C-PVP NMBA 2% hydrogel. As shown in table 6.2 for hydrogels prepared with (1-2)% crosslinking agent, the values of G(X) ≈ G(S) or G (X) > G (S). If G(S) > G(X), degradation processes are assumed to prevail. For hydrogels prepared with reduced concentration of crosslinking agent (3_C-PVP_NMBA 0.5%), crosslinking processes predominated compared to degradation processes, the highest value of parameter G(X) being determined for the hydrogel prepared at the dose of 7.5 kGy. In the range (10-25) kGy, the radiation cross-linking yields remained almost constant, but at the dose of 40 kGy, they decreased. In previous studies, regarding the processing with γ-radiation of natural polymers in solid state, it has been shown that the radiation dose rates as well as the concentration of polymers are critical parameters that influence the final result of the irradiation process [18, 22]. The irradiation conditions influence the result of the irradiation process, therefore low dose rates will lead to increased of oxidative processes which at the same time produce degradation of the polymer macromolecules during irradiation.

Table 6.2. Radiation crosslinking yields G(X) and degradation G(S) computed for C-PVP hydrogels prepared with the addition of crosslinking agents

Sample	G(X) mol J^{-1}					
	Dose (kGy)					
	5	7,5	10	12,5	25	40
1_C-PVP_NMBA 2%	1,07	0,94	1,12	1,14	0,25	0,07
2_C-PVP_NMBA 1%	1,01	0,54	0,37	0,20	0,12	0,06
3_C-PVP_NMBA 0,5%	0,23	0,25	0,13	0,10	0,14	0,08

Sample	G(S) mol J^{-1}					
	Dose (kGy)					
	5	7,5	10	12,5	25	40
1_C-PVP_NMBA 2%	1,09	0,96	1,15	1,16	0,25	0,08
2_C-PVP_NMBA 1%	1,60	0,85	0,59	0,31	0,19	0,09
3_C-PVP_NMBA 0,5%	0,15	0,16	0,09	0,03	0,09	0,05

- **Swelling degree**

For hydrogels prepared with (1-2)% NMBA, no dependence of the swelling degree was observed depending on the absorbed dose. For the C-PVP hydrogel cross-linked with e-beam in the presence of 0.5% NMBA, a significant increase in the degree of swelling up to 11 000% was observed. For hydrogels prepared with 0.5% NMBA, contrary a dependence of the swelling degree on the absorbed dose was observed, at 40 kGy, GG (%) decreased to 4000%.

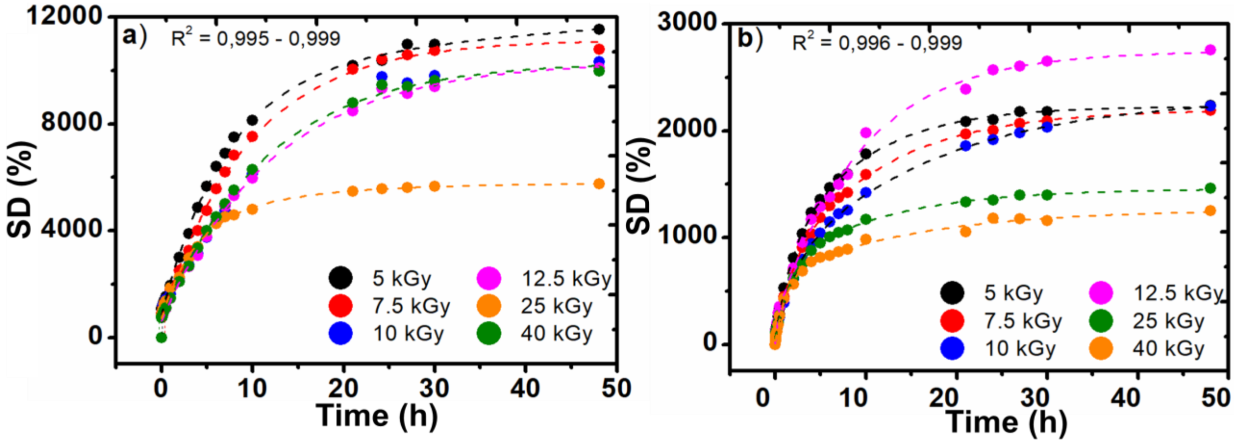


Figure 6.4. Swelling degree as a function of the immersion time and the absorbed dose in deionised water (a) and (b) in PBS (37°C, pH=7,4) for hydrogels prepared with 0,5% NMBA

The hydrogel showed stability higher than 48 hours in PBS solution, but the total swelling power was reduced to 2500%. In this case, GG(%) decreased depending on the applied dose, at (5–10) kGy, (25–40) kGy due to the increase of the crosslinking points in the hydrogel network. The same behavior was also identified when the network parameters were investigated, more precisely the M_C decreased, and the crosslinking density increased. When the C-PVP solution was irradiated at 12.5 kGy, GG (%) and M_C increased. In the range (10–12.5) kGy, the properties of the hydrogel were similar. The values of the elastic modules (G') were almost similar (2800 Pa at 10 kGy and 2500 Pa at 12.5 kGy). The reduction of module G' may be the consequence of a higher absorption power, if the absorbed dose increased twice, the elastic character of the hydrogels increased accordingly. The significant decrease in the swelling capacity of the hydrogels in the PBS solution compared to the values determined in deionised water is due to the osmotic pressure caused by the unequal distribution of ions in the environment and in the hydrogel network. The ions attached to the macromolecular chain of polymers remain trapped inside the hydrogel network. When swelling occurs in pure water, maximum osmotic pressure develops and maximum swelling values are obtained [23].

- **Rheological analysis**

The values of the elastic and viscous modules depended on the concentration of NMBA, on the composition of the hydrogels, as well as on the absorbed dose. Hydrogels prepared with 2% NMBA show better rheological properties (12000 Pa at 10 kGy), but the swelling experiments showed that a weak hydrogel was obtained in terms of stability for 48 h in a swelling environment. Usually, with increasing of polymers and crosslinking agent concentration, the phenomenon of chain overlap increases, which favours crosslinking processes. For such systems, higher viscosity decreases the mobility of the polymer chains, reducing the possibility for the macroradicals to react each other to form stable chemical bonds [24]. The results obtained from the sol-gel analysis, the degree of swelling and from the rheology experiments, show that the 3_C-PVP_0,5% NMBA hydrogel has the best properties. The values of the G' modulus varied between (1800-8400) Pa, and the best swelling properties in PBS at 37°C were found for those hydrogels crosslinked with doses starting from 7.5 to 12.5 kGy.

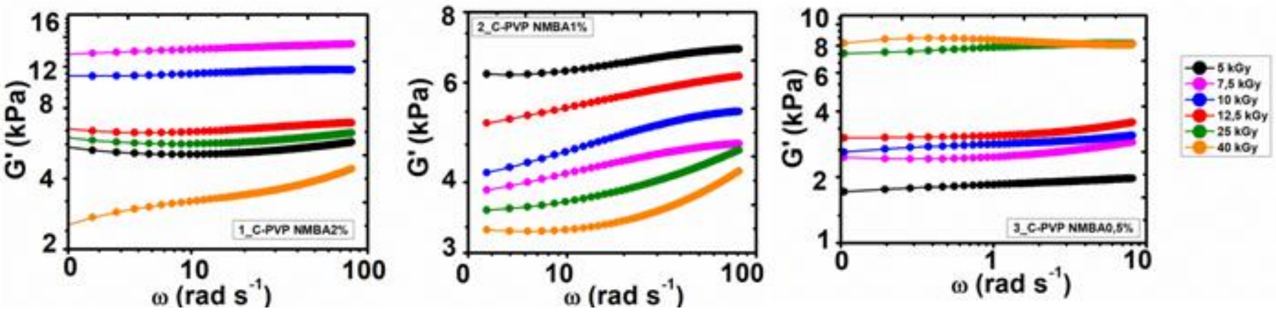


Figure 6.5. The variation of G′ vs. angular velocity (ω) the absorbed dose

In conclusion, superabsorbent hydrogels with different properties can be synthesized taking in account the overall e-beam irradiation parameters. Given the elasticity of the prepared hydrogels, their use in the medical field can be expected. The elastic modulus of C-PVP hydrogels matches that of human skin and soft tissue, being relevant for the successful application of this hydrogel as a wound dressing or in tissue engineering of soft tissues (the elastic modulus of human skin varies between (500-1000) Pa [25].

The manufacture of stable C-PVP hydrogels with constant elastic properties (G′ = 1800 - 8000 Pa), capable of absorbing a large amount of aqueous fluids, similar to physiological liquids, can be achieved by e-beam crosslinking in the absence of oxygen and by adding small amount of water soluble crosslinking agents.

- **Crosslinking density**

The network parameters of the hydrogels obtained in the presence of NMBA showed reduced M_C values, correlated with high values of the elastic modules (G′). If the concentration of NMBA is lower, the polymeric mixtures of collagen and PVP can be crosslinked by e-beam irradiation at moderate doses, close to the legally accepted dose of sterilization (25 kGy), so as to obtain hydrogels with optimum swelling and rheological properties. As the number of crosslinking points increases, the value of the G′ modulus also increases, and the parameters M_C and ξ decrease, indicating the formation of a hydrogel with a dense structure. In order to produce a superabsorbent hydrogel from collagen and PVP with demonstrated stability in PBS at 37°C for at least 48 hours and a degree of swelling over 2000%, the average molecular mass between two crosslinking points (M_C) must be between 60 and 66 kg • mol^{-1}, and the mesh size (ξ) is (53–54) nm. The results indicate that the network parameters of the 3_C-PVP_NMBA 0.5% hydrogel can be controlled by varying the irradiation dose, which was not observed for hydrogels prepared with (1–2)% NMBA.

Table 6.7. Experimental values of G′, G′′, M_C, Ve and ξ [26]

	3_C-PVP_NMBA 0,5%				
Dose (kGy)	$\bar{G}'$ **(kPa)**	$\bar{G}''$ **(kPa)**	M_C (kg mol^{-1})	**Ve** (mol m^{-3})	**ξ** (nm)
5	1,8	0,13	71	14,14	59
7,5	3,2	0,51	45	22,14	47
10	2,8	0,52	60	17,14	53
12,5	2,5	0,56	66	15,55	54
25	7,8	1,03	24	41,50	27
40	8,4	1,51	25	40,10	26

- **FTIR analysis**

For 3_C-PVP NMBA 0.5% hydrogel irradiated with (5-40) kGy doses from FTIR spectra (figure 6.6) it was observed that the intensity of the amide II band was maintained when the hydrogel was crosslinked with 25 kGy. The amide band I shifted to 1668 cm^{-1} (toward larger wave numbers), but the intensity corresponding to this band was maintained; in the region (3700-2700) cm^{-1} the intensity of the bands decreased, and the characteristic peak was shifted from 3300 cm^{-1} to 3360 cm^{-1}; the shifting of main peaks to larger wavenumbers may be associated with the scission of the hydrogel network chain if the polymer mixture is exposed at doses greater than 25 kGy.

- **Scanning electron microscopy (SEM)**

The structure of the hydrogels varied according to the irradiation dose and presented a more compact form at low doses of irradiation and macroporous structure at high doses of irradiation. With the increase of the irradiation dose, at 25 kGy and 40 kGy, the structure of the hydrogel has a macroporous structure with large pores (about 50 µm diameter). At all irradiation doses, at very large magnifications of the image, interconnected crystalline formations that adhere to the macroporous surface of the hydrogel were identified. These crystalline formations may originate from PBS which has been used to swell hydrogels. For the crosslinked hydrogel with a dose of 10 kGy, a well-defined porous structure with interconnected pores of variable size is distinguished.

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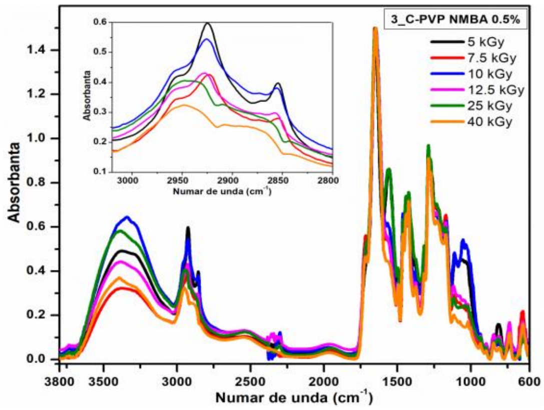


Figure 6.6. FTIR spectra for 3_C-PVP_NMBA 0,5% hydrogels

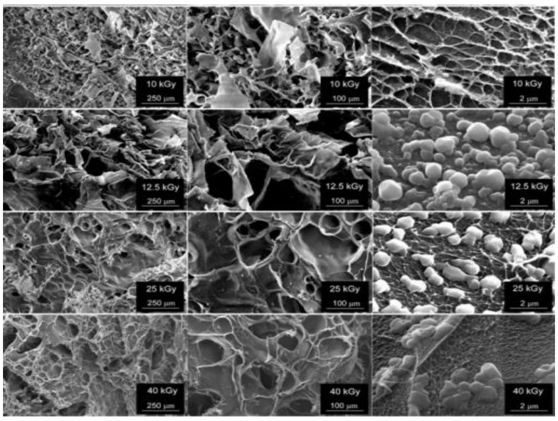


Figure 6.7. SEM images for 10 kGy and 40 kGy crosslinked C-PVP hydrogels [26]

CHAPTER 7. E-BEAM RADIATION CROSSLINKING OF COLLAGEN/PVP/PEO THREE-COMPONENT HYDROGELS OBTAINED IN ARGON ATMOSPHERE

This chapter presents the obtaining of a three-component superabsorbent hydrogels prepared form collagen, PVP and polyethylene oxide (PEO). A comparison within collagen/PVP and collagen/PVP/PEO hydrogels obtained in argon atmosphere is presented. Figure 7.1 shows the good elasticity and shape retention for both types of hydrogels, with and without PEO. The aim of the study was to obtain a hydrogel with high elastic properties and prolonged stability in physiological environments.

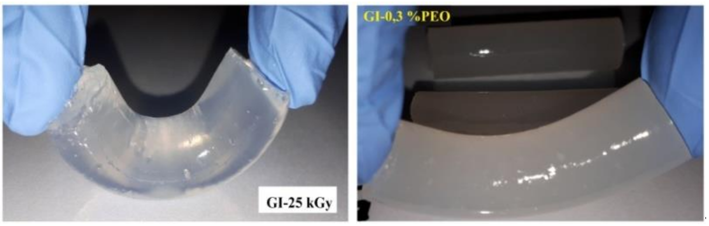


Figure 7.1. The appearance of hydrogels with and without PEO after removal from the containers in which they were irradiated

7.1. Characterization of hydrogels obtained in an inert atmosphere

- **Sol-gel analysis**

The gel fraction G (%) decreases with the irradiation dose for the hydrogel prepared without PEO (Figure 7.2).At a maximum dose of 25 kGy, the decrease is up to 77%. For the PEO hydrogel, G(%) increases with the irradiation dose, up to 84%. For the hydrogel without PEO, G(X) and G(S) increase with increasing dose, and for the hydrogel with PEO, G(X) and G(S), there is no irradiation dose dependence. In the literature [27] it is shown that for materials with a ratio G(S):G (X) < 1, the crosslinking reaction is favoured, and if the ratio G(S): G(X) > 1, the degradation processes are favoured. From this ratio (table 7.1), it appears that for the product without PEO, the cross-linking processes are predominant, and for the hydrogel with PEO, there is a slight tendency towards degradation reactions.

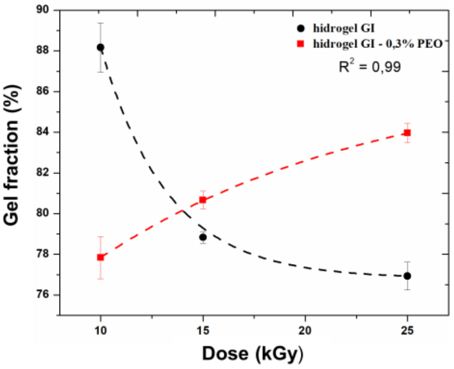


Figure 7.2. Gel fractions vs. absorbed dose

Table 7.1. Radiation chemical yields of crosslinking G(X) and degradation G(S)

Sample	G(X) µmol J⁻¹			G(S) µmol J⁻¹			G(S):G(X)
	Dose (kGy)						
	10	15	25	10	15	25	
GI	0,44	0,46	0,68	0,38	0,40	0,58	0,85-0,87
GI – 0,3 % PEO	0,80	0,64	1,01	0,87	0,69	1,11	1,08-1,09

- **The degree of swelling and stability of hydrogels in physiological environments**

The degrees of swelling and structural stability of the hydrogel are two essential features for their use in tissue engineering. The stability and swelling capacity of hydrogels with and without PEO have been studied in deionised water and phosphate buffer saline (PBS) which mimics the physiological state (pH, osmolarity and ion concentration) of the human body.

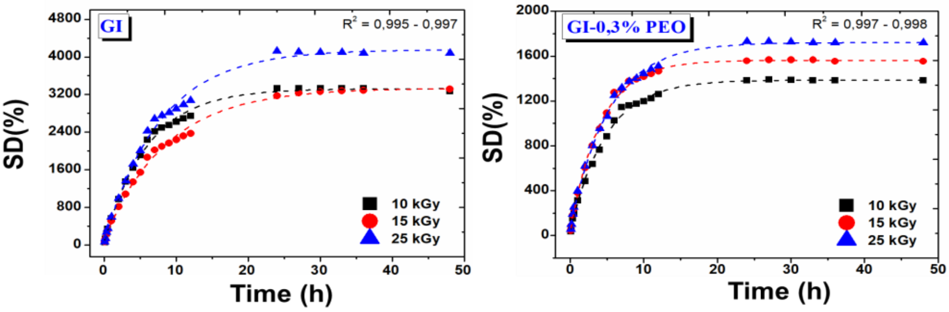


Figure 7.3. Swelling degree in deionised water (37°C) - GI hydrogel

For the hydrogel without PEO, GG(%) obtained at 25 kGy, has the value of 4000%, and for the one with 0.3% PEO, it is about 1600% (approximately 2.5 times smaller), which shows that by increasing the concentration of polymers in the composition of the hydrogels and increasing the crosslinking density, the macromolecular network of the hydrogel becomes more dense and the diffusion of the water molecules in the swelling environment is prevented. The swelling equilibrium is reached after 16 hours and the physical form is stable up to about 50 hours. For the potential use of these hydrogels as dressings for the rapid healing of the wounds of the skin, its stability has been tested in various environments that simulate the pH of healthy skin and in the presence of an infected wound. In the case of a wound, the so-called "exudates" is formed of nutrients, electrolytes, inflammatory substances, leukocytes, proteins which in the presence of the hydrogel are absorbed through the swelling process, leading to healing. The use of an inadequate hydrogel (dressing) does not exclude the risk of infection and chronicization of the wound. Swelling and stability experiments for the synthesized hydrogels were performed in solutions with pH = 5.4 (healthy skin) and pH = 9.4 (infected wound) [28].

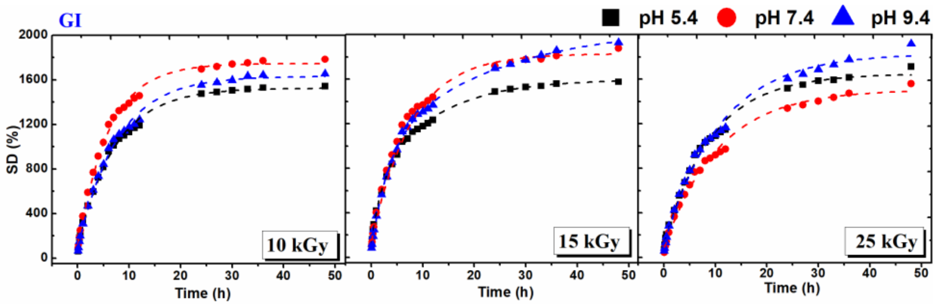


Figure 7.4. Swelling degree as a function of the time and pH (37°C)

In the case of determinations carried out in solutions with pH (5.4 - 9.4), the degree of swelling has decreased to about 1750%, but the stability and the reaching time of equilibrium were stable. This finding indicates the use of these hydrogels in obtaining of dressings intended to cure more or less infected wounds. Regarding the variation of the degree of swelling over time and in relation to the pH, there is no obvious dependence, but the equilibrium of swelling is almost constant (1650-1750)%.

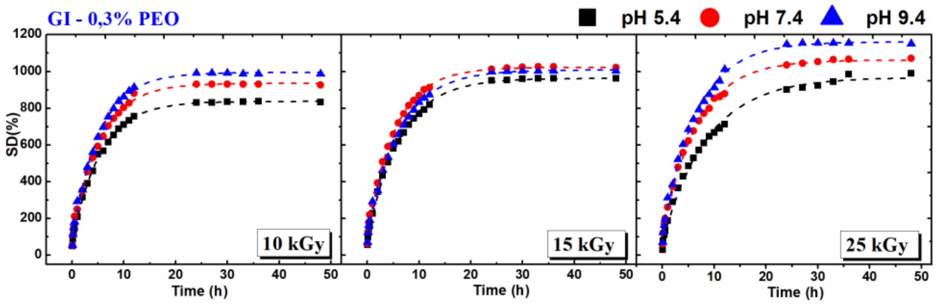


Figure 7.5. Swelling degree as a function of the time and pH (37°C)

Addition of PEO decreases GG% at equilibrium, regardless of pH and dose (figure 7.8). For example, for the hydrogel obtained at the dose of 25 kGy, GG% is about 1150%, which means that 1 g of dry hydrogel absorbs about 12 g of liquid similar to physiological fluids. From the graphical representation it is observed that for the neutral and basic environment, GG% at equilibrium is higher than the corresponding one in acidic environment. This is due to the activation of the deprotonation mechanisms of the donor proton groups (NH, COOH, OH), as a consequence of the disintegration of the inter-molecular or intra-molecular crosslinking points in the basic environment, which ultimately leads to the increase of the mesh size of the hydrogel network structure [29]. The most important interaction occurs through the H bonds formed following the exchange of protons from donor to acceptor groups. For example, PVP and PEO are proton acceptor polymers and form strong H bonds with proton donor polymers such as collagen and AA, favouring the association of component polymers by forming stable chemical bonds, as well as increasing the structural stability of the hydrogel [30, 31].

- **Swelling kinetics in deionized water**

The swelling process of a hydrogel consists of a *solid* → *liquid* transition, without dissolving or dissociating the hydrogel in the aqueous environment. The liquid enters into polymer network through diffusion, leading to its swelling by different mechanisms. In Fickian diffusion, the hydrogel polymer chains have a high degree of mobility and relaxation, which makes the water easier to enter in the relaxed network (n = 0.50). In non-Fickian diffusion, the diffusion process is very fast compared to the chain relaxation processes (0.50 <n <1). In this case, the polymer chains in the hydrogel are not sufficiently mobile so as to allow a rapid diffusion of water into the hydrogel structure. Another case of diffusion encountered is type III or abnormal diffusion that occurs when the rate of water diffusion is approximately equal to the relaxation rate [32]. Based on these considerations for the prepared hydrogels, the diffusion mechanisms were studied both in deionised water and under different pH conditions.

Table 7.2. Diffusion parameters – hydrogel without PEO in deionised water

Dose	Diffusion parameters, I		Diffusion parameters, II		Diffusion parameters, III	
(kGy)	n	R^2	n	R^2	n	R^2
10	0,62	0,997	0,68	0,989	0,23	0,989
15	0,59	0,998	0,72	0,988	0,30	0,998
25	0,55	0,996	0,78	0,980	0,27	0,980

From table 7.2 it is observed that in the time interval (5-180) minutes, n > 0.5 which means that the diffusion is characterized by a non-Fickian mechanism and decreases with the dose, being minimum at 25 kGy; in the time interval of (240-420) minutes, n> 0.5, so a non-Fickian mechanism is present, but n, increases with the dose, being maximal at 25 kGy, as a result of the relaxation of the polymer chains and probably of sufficient hydration of the crosslinking points formed by irradiation; within the time period (480-720) minutes, n <0.5, pseudo-Fickian diffusion is shown and means that the diffusion is reduced as a result of reaching equilibrium [33] and there is no dependence on the irradiation dose and in good approximation n it is constant.

Table 7.3. Diffusion parameters – GI-0,3% PEO hydrogel in deionised water

Doza	Diffusion parameters, I		Diffusion parameters, II		Diffusion parameters, III	
(kGy)	n	R^2	n	R^2	n	R^2
10	0,45	0,982	0,76	0,998	0,15	0,949
15	0,50	0,982	0,78	0,996	0,22	0,898
25	0,50	0,982	0,72	0,994	0,26	0,992

From table 7.3 it is observed that in the interval of (5-150) minutes, n < 0.5, Fickian mechanism, n increases very little from the dose of 10 kGy to the dose of 15 kGy and then remains constant; within (180-450) minutes, n > 0.5, non-Fickian mechanism, n has no clear dose dependence and in good approximation can be considered constant; in the (480-720) minutes, n < 0.5, pseudo-Fickian mechanism. The addition of PEO increases the degree of mobility and relaxation of the polymer chains in the hydrogel, leading to improved diffusion. Figure 7.6 shows the swelling kinetic curves for the GI hydrogel in buffer solutions with pH = 5.4 - 9.4.

From the diagrams it is observed that in the pH range (5.4 - 9.4) and the dose range of (10-25) kGy, diffusion occurs in two stages, following a pseudo-Fickian or Fickian process. For the dose of 15 kGy, in the pH range (7.4 - 9.4), so in neutral and weak alkaline environment, n is slightly greater than 0.5, indicating a non-Fickian mechanism. For the hydrogel prepared with 25 kGy, in the interval of (5 - 120) minutes the diffusion runs pseudo-Fickian, and in the interval (180 - 480) min, the diffusion is Fickian. The process of swelling of the hydrogels is dependent on the contribution of the diffusion medium and the relaxation of the polymer chain.

In the hydrogels containing ionisable functional groups, electrostatic repulsions occur, which leads to the extension of the chains that affect the relaxation of the macromolecular chains. Increasing the degree of crosslinking does not significantly alter the ionization of the functional groups, which means that the relaxation of the macromolecular chains is reduced and the parameter n is lower [34]. Figure 7.7 shows the swelling kinetic curves for the GI-0.3% PEO hydrogel. It is observed that for this hydrogel, in the pH range (5.4 - 9.4), the diffusion mechanism proceeds in two time-marked steps. Regardless of the dose, the two stages result from a non-Fickian mechanism. In weak acid and neutral medium, (n) increases with the irradiation dose for the first stage and decreases for the second stage. For alkaline environment, (n) decreases with dose for the first stage and remains constant for the second stage.

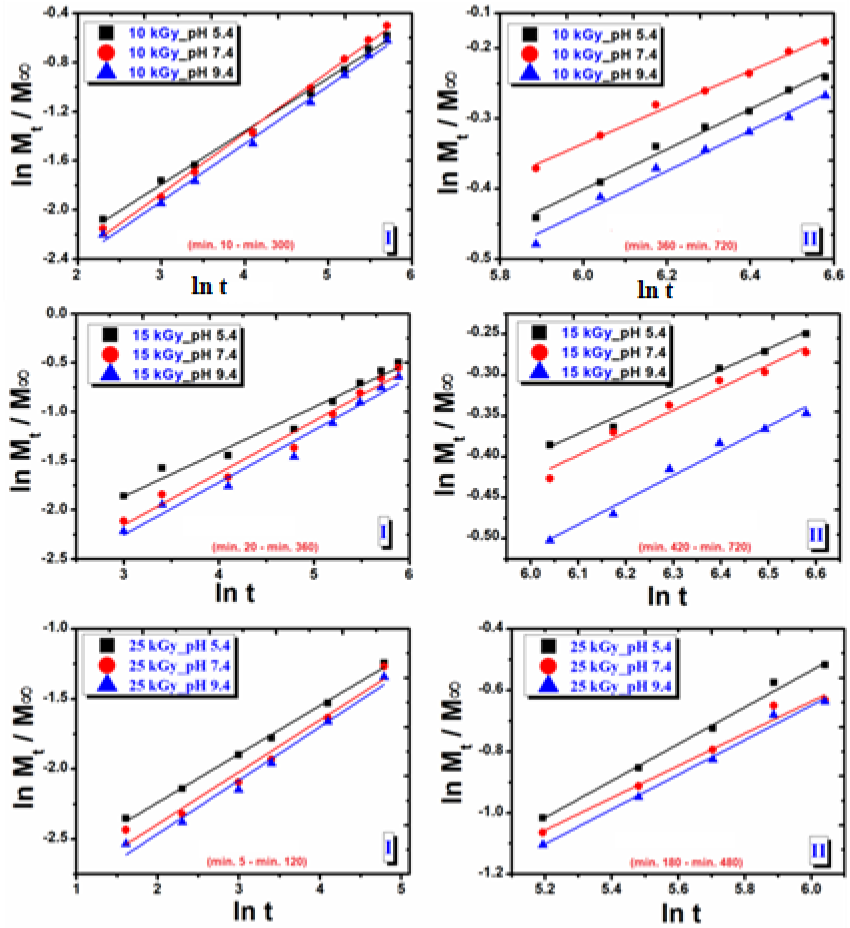


Figure 7.6. Swelling kinetic curves for the GI hydrogel in solutions with pH = 5.4 - 9.4

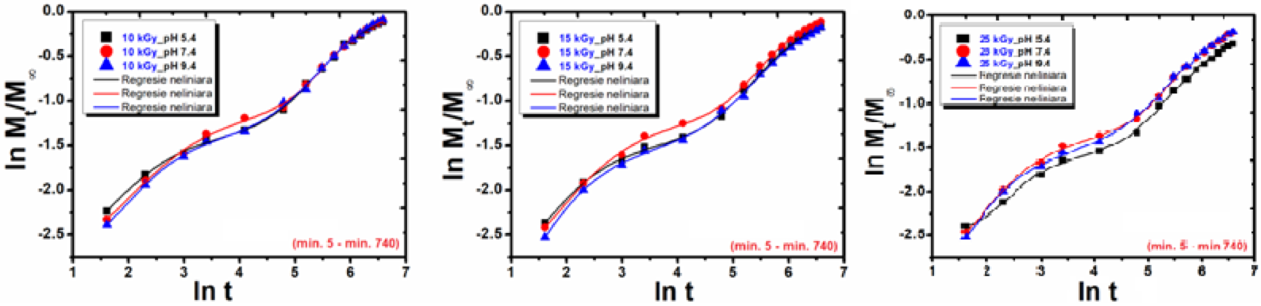


Figure 7.7. Swelling kinetic curves - GI-0.3% PEO hydrogel in buffer solutions with pH = 5.4 - 9.4

- **Rheological analysis**

The rheological behavior of hydrogels prepared without addition of PEO is shown in figures 7.8 and 7.9 It is observed that with the increase of the irradiation dose the increase of module G' (1600 - 6000) Pa and the decrease of G' modulus (173 - 60) Pa occur. The 25 kGy dose (sterilization dose) increases approximately 4 times the value of G' compared to its value at the dose of 10 kGy.

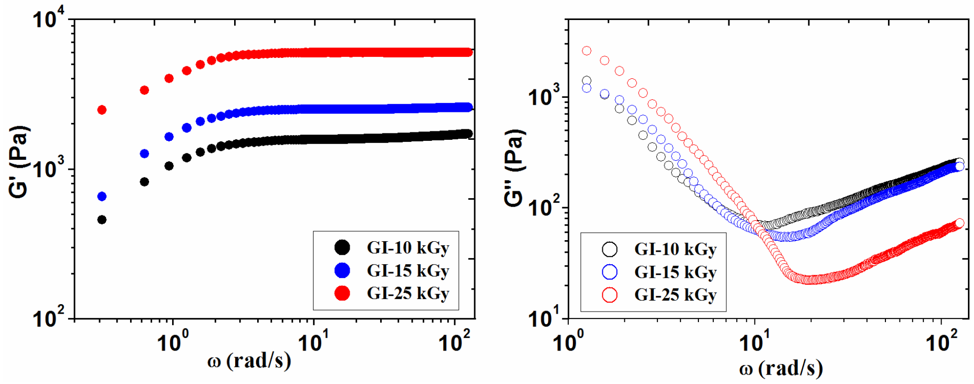


Figure 7.8. Variation of G' and G" depending on the angular frequency (ω) and the absorbed dose (hydrogel prepared without PEO)

From the relation between the G' and G'' modules it can be concluded that the hydrogel is elastic when is obtained with high doses (25 kGy). At the molecular level, a predominantly elastic rheological behavior (G' > G") is associated with rapid and reversible changes in the length, shape or orientation of the chains that appear when a force is applied to a polymer chain. The increased viscosity is given by slow, irreversible processes of energy dissipation, which appear as a consequence of the movement of macromolecules [35].

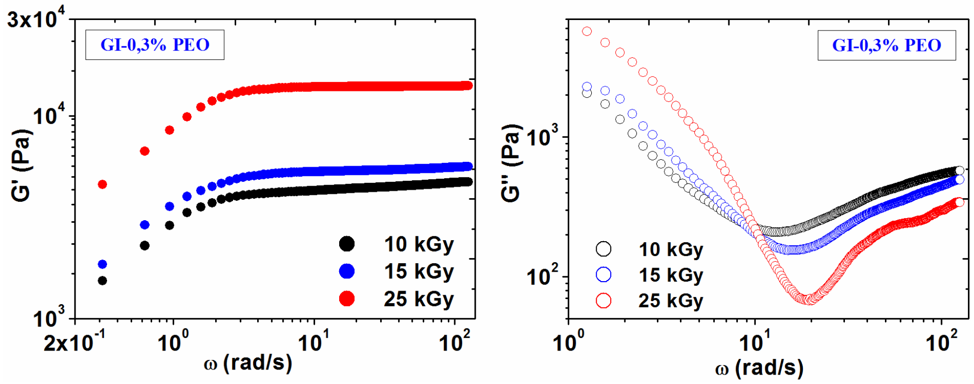


Figure 7.9. Variation of G’ and vs. angular frequency (ω) and the absorbed dose (hydrogel prepared with PEO)

Physically, PEO chains remain attached to the main chains of the constituent polymers and act as a physical crosslinking agent [36]. By adding 0.3% PEO in the hydrogel composition, the elastic modulus of the hydrogel increased 3 times, compared to that without PEO and is between (4500-14000) Pa. For potential soft tissue healing applications, the elastic modulus of a polymeric matrix in the form of a hydrogel should be between (10^3 - 10^4) Pa [37]. For applications aimed the rapid healing of skin lesions, such dressing should have rheological characteristics similar to those of the skin and human dermis, which has an elastic modulus (G’) in the range (500 - 1000) Pa [25].

- **Crosslinking density (Ve)**

The network structure of a superabsorbent hydrogel is characterized by a high elasticity and capacity of the pores to expand in an aqueous environment (150 - 1500) times greater than their size in the dry state. Pore size is the major factor controlling the swelling degree of the hydrogels, which is related to the crosslinking density and the type of functional groups immobilized on the polymer chain [32]. The experimental values determined for the parameters describing the macromolecular structure of GI and GI-0.3% PEO hydrogels are presented in tables 7.4 and 7.5. The values of the elastic modules were used to determine the characteristic parameters of the hydrogels structure.

Table 7.4. Experimental values of G’, G’’, M_C, Ve and ξ (GI hydrogel)

Dose (kGy)	$\bar{G}'$ (Pa)	$\bar{G}''$ (Pa)	M_C (kg mol^{-1})	V_e (mol m^{-3})	ξ (nm)	ρ (kg m^{-3})	$(\nu)_{2,r}{}^{2/3}$	$(\nu)_{2,s}{}^{1/3}$
10	1643	174	113	8,84	60	1001	0,2605	0,2873
15	2536	154	72	13,89	50	1005	0,2605	0,2769
25	5977	60	30	33,75	33	997	0,2644	0,2709

Tabelul 7.5. Experimental values G’, G’’, M_C, Ve and ξ (GI-0,3% PEO hydrogel)

Dose (kGy)	$\bar{G}''$ (Pa)	$\bar{G}''$ (Pa)	M_C (kg mol^{-1})	V_e (mol m^{-3})	ξ (nm)	ρ (kg m^{-3})	$(\nu)_{2,r}{}^{2/3}$	$(\nu)_{2,s}{}^{1/3}$
10	4530	452	61	16,38	32	1003	0,2802	0,3968
15	5447	382	51	19,56	29	1004	0,2795	0,4000
25	14 000	248	19	51,73	19	1007	0,2826	0,3837

It is observed that Mc and ξ decrease with increasing of irradiation dose while the crosslinking density increases, suggesting a stronger crosslinking for the 25 kGy crosslinked hydrogels. The values of the network parameters bring important information regarding the use of hydrogels and help optimize the properties for a certain type of application, such as: dressings or controlled release of some drugs. Also, their evaluation according to the radiation dose allows to establish with certainty whether these types of systems can be obtained at a dose of irradiation which will also ensure the sterilization of the hydrogel. For each of the two systems (with and without PEO), it is observed that at the 25 kGy dose, the values of the G’ modules are high and are similar to those characteristic of soft tissues

- **FTIR analysis**

The composition of the GI and GI hydrogels - 0.3% was evaluated by FT-IR spectroscopy, compared to the FT-IR spectra of the single component polymers.

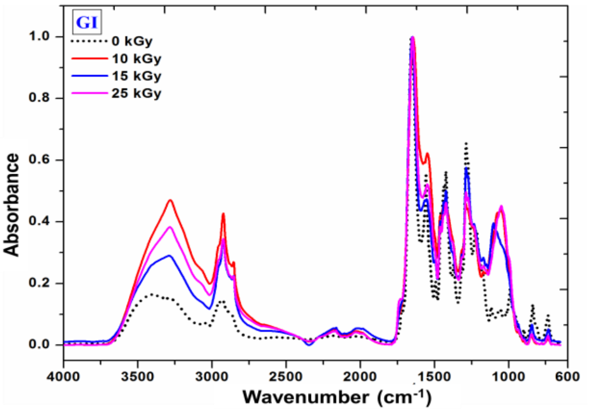


Figure 7.10. FT-IR GI hydrogel spectra

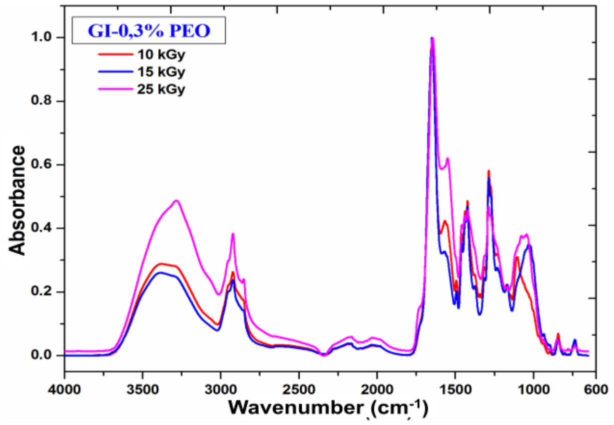


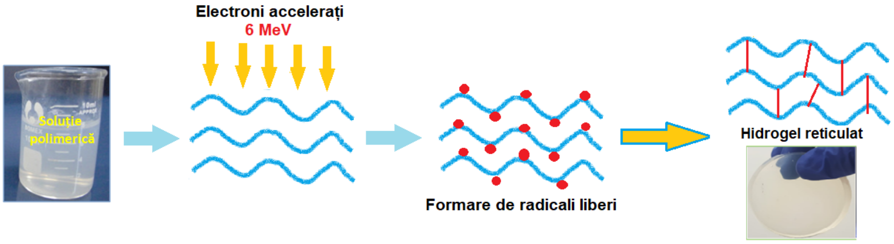
Figure 7.11. FT-IR spectra of GI-0,3% PEO hydrogel

For the GI hydrogel in the FT-IR spectra (Figure 7.10) it was observed that in the range (3700-2700) cm^{-1}, the peak intensity increased and shifted to larger wavenumbers, from 3290 cm^{-1} (10 kGy), at 3324 cm^{-1} (15 kGy). After irradiation with 25 kGy, a displacement to smaller wavenumbers occurs at 3302 cm^{-1}; the shift to larger wave numbers is due to the decrease in chemical bond length as a consequence of increased intra- and inter-molecular interactions through H; the amide band III (1290 cm^{-1}) changes its position to smaller wavenumbers with approximately 20 cm^{-1} and decreases slightly in intensity with increasing dose. This band is characteristic of proteins such as collagen and provides information on their secondary structure. At 25 kGy, a new band at 1053 cm^{-1} appears which is associated with the introduction of the C–OH groups specific to primary alcohols, as a consequence of the oxidative processes triggered at this irradiation dose [17].

From the FT-IR spectra recorded for the GI-0.3% PEO hydrogel (figure 7.11), the following characteristic peaks were identified and attributed as follows: in the range (3700 - 2700) cm^{-1} the absorption bands become large. and increases the intensity at the dose of 25 kGy; the peak is shifted to smaller wavenumbers from 3352 cm^{-1} (10 kGy) to 3311 cm^{-1} (25 kGy); the collagen bands maintain their position; after irradiation with 15 kGy and 25 kGy the band is shifted from 1104 cm^{-1} to 1046 cm^{-1} and is also associated with the occurrence of OH groups belonging to a primary alcohol, probably due to an oxidation reaction, which occurs without scission of the C–C bonds and transformation of secondary OH groups into ketones; it is observed that after irradiation with 15 and 25 kGy, a reduced intensity shoulder appears in the FT-IR spectrum at 1735 cm^{-1}, characteristic to ketone

CHAPTER 8. EFFECT OF PEO CONCENTRATION ON THE E-BEAM SYNTHESIS OF COLLAGEN/PVP/PEO SUPERABSORBENT HYDROGELS

In this chapter, hydrogels with superabsorbent properties were obtained starting from the previously optimized hydrogels formulation. As for the previously prepared hydrogel formula, the best results were obtained in the dose range (7.5 - 12.5) kGy, irradiation doses insufficient for concomitant assay of the crosslinking reaction. In order to obtain and the sterilization of the final product, it was considered necessary a modification of the previous hydrogels formulation to allow the irradiation with higher doses and to reach the two findings: cross-linking and sterilization. In order to illustrate the crosslinking process, in the scheme 8.1 is the basic principle of the crosslinking reaction initiated under the e-beam action is presented. By irradiation of a polymeric aqueous solution, free radicals are formed on the polymer chains following the radiolysis process, and as a result of the recombination reactions between the macroradicals, solid materials with compact structure are formed.



Scheme 8.1. General scheme of hydrogels formation by irradiation

8.1. Characterization of collagen/PVP/PEO hydrogels

- **Sol-gel analysis**

The G(%) increases with increasing of irradiation dose and decreases with increasing PEO concentration. From the above curves it appears that for any dose, the recommended concentration range is (0.2 - 0.5)%. The higher concentration of polymers, the polymeric system has a higher viscosity and consequently a reduced mobility of the macroradicals resulting after irradiation. The increase of the PEO concentration in these systems over 0.5% does not favour the crosslinking processes, also at concentrations below 0.2% PEO, the cross-linking does not prevail.

Table 8.3. Radiation chemical yields of crosslinking G(X) and degradation G(S)

Sample	**G(X) μmol J^{-1}**			**G(S) μmol J^{-1}**			
	Dose (kGy)						
	10	**15**	**25**	**10**	**15**	**25**	**G(S):G(X) (25 kGy)**
Gl – 1% PEO	0,22	0,20	0,36	0,18	0,17	0,30	0,83
Gl – 0,5% PEO	0,41	0,48	0,65	0,30	0,36	0,48	0,74
Gl – 0,25% PEO	0,42	0,45	0,84	0,07	0,08	0,15	0,17
Gl – 0,1% PEO	0,26	0,26	0,34	0,37	0,37	0,48	1,41

For systems prepared with (0.25 - 0.5)% PEO, G(X) and G(S) increase with dose and G(X)>G(S), which shows the predominance of the crosslinking processes, opposite to the degradation. In contrast, in the systems prepared with 0.1% PEO, G (S) > G (X), the degradation processes were predominant. For hydrogels prepared with 0.3% PEO in inert atmosphere of Ar, G(X) ≈ G(S), (presented in Chapter 7). In this case it was established that successive centrifugation → degassing operations are sufficient to obtain higher quality hydrogels with G(X) >> G(S), so as to obtain a hydrogel with properties controlled by irradiation with e-beams. Usually in practice the following cases are encountered: G(X) >> G(S), the molecular mass increases continuously due to the continuously crosslinked bonds; G(X) ≥ G(S), G(S) at the end of the reaction will equal G(X) and the molecular mass will register a inflection point, and the general reaction will result from the crosslinking → scission; G(X) ≪ G(S), degradation occurs continuously [27].

- **Rheological analysis**

Regardless of the PEO concentration added to each system, G′ modulus increased with the irradiation dose and is constant in the investigated angular frequency range. At the irradiation dose of 25 kGy, the G′ modulus had values between (3241-9816) Pa and depends on the added PEO concentration, having the highest value at 0.25% PEO. G′ modulus increases depending on the irradiation dose, but at concentrations greater than 0.35% PEO introduced in the system, this parameter decreases sharply. In this case, a concentration of 0.35% PEO could be considered optimal, for the significant improvement of the elastic properties of such systems. For all samples across the frequency range studied, G′ is greater than G″, shows that the hydrogels exhibit a predominantly elastic behavior specific to the solid materials with elastic properties [38].

- **The swelling degree and the stability of hydrogels in simulated physiological environments**

Figure 8.2 shows the variation of the swelling degree in deionised water at 37°C depending on the immersion time and the irradiation dose. The SD(%) of the hydrogels prepared with different concentrations of PEO, depends on the irradiation dose and their composition, generally increases with the irradiation dose and decreases with the PEO concentrations. For example, hydrogels prepared with (0.5-1)% PEO showed a swelling degree in the range (3000 - 7100)%, which means that these hydrogels are capable of incorporating an amount of ~ (30 – 71) *g water/g hydrogel* in their structure. For hydrogels prepared with (0.1 –0.25)% PEO the SD(%) is between (1500 - 5500)%, which means that these gels include a smaller amount of water, of ~ (15 - 55) *g water/ g hydrogel*.

GG(%) determined in solutions with different pH is reduced compared to GG(%) determined in deionised water and is in all cases higher under neutral pH conditions. In different pH solutions, the investigated hydrogels showed stability over 48 hours and reached equilibrium after 8 - 10 hours. All hydrogels have a liquid absorption capacity in the pH range = 5.4 - 9.4 of ~ (10-20) g *liquid / g hydrogel*. The swelling is favourable in weak acidic environment for the hydrogel produced with 25 kGy. If the PEO concentration is further reduced, the swelling is increased in neutral and weak basic environment. In this case, the hydrogels reach equilibrium much faster, compared to the other systems, less than 6 hours, in a weak acid and neutral environment.

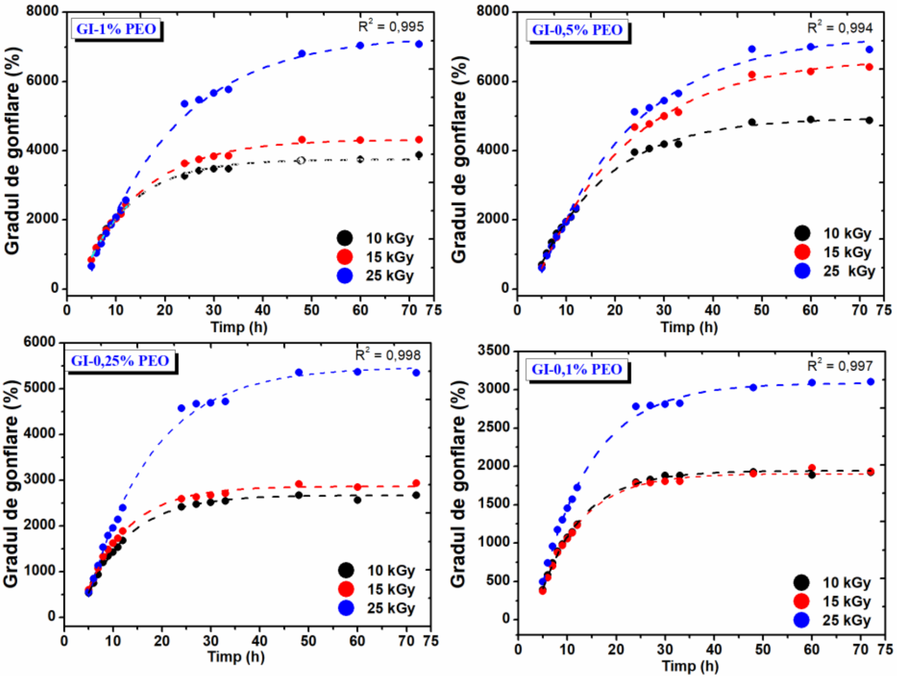


Figure 8.2. Degree of swelling in deionised water (hydrogels prepared with (0.1-1)% PEO

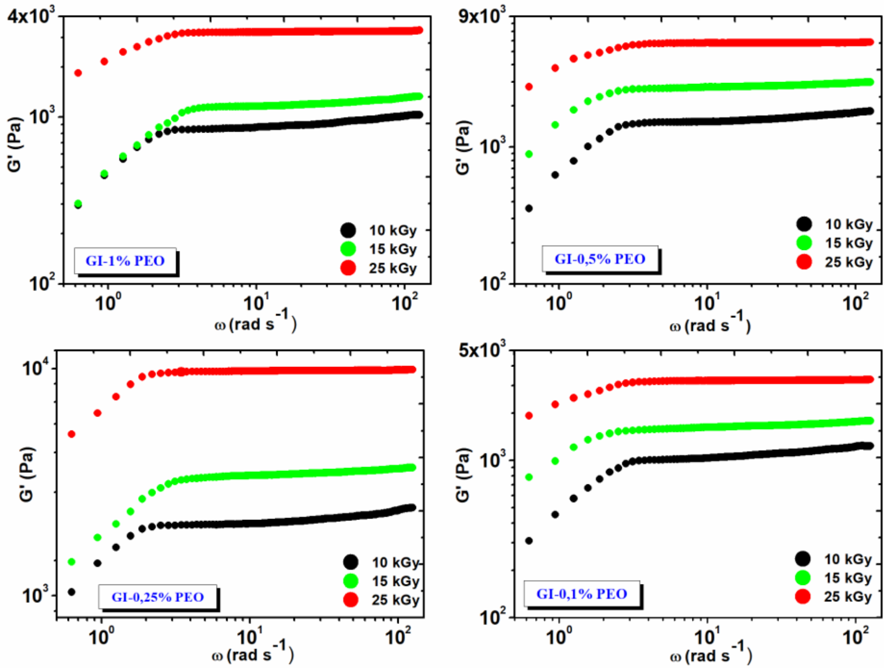


Figure 8.3. The variation of G′ depending on the angular frequency (ω) and the absorbed dose

- **Hydrogels network structure**

The parameter M_C decreases with increasing of irradiation dose for the compositions with (0.25 -1%) PEO, and the crosslinking density (Ve) increases with the irradiation dose, being maximum at 25 kGy for system prepared with 0.25% PEO. Parameter ξ decreases with irradiation dose for all concentrations.

Table 8.5.Experimental values for G′, G″, M_C, Ve and ξ

				Hydrogel GI-1% PEO				
Dose	$\bar{G}''$	$\bar{G}''$	M_C	V_e	ξ	ρ	$(\nu)_{2,r}{}^{2/3}$	$(\nu)_{2,s}{}^{1/3}$
(kGy)	**(Pa)**	**(Pa)**	**(kg mol⁻¹)**	**(mol m⁻³)**	**(nm)**	**(kg m⁻³)**		
10	937	187	228,54	0,45	72	1033	0,2419	0,3454
15	1253	184	163,95	0,62	63	1028	0,2412	0,3338
25	3260	85	54,98	1,87	42	1028	0,2432	0,2889
				Hydrogel GI-0,5% PEO				
Dose	$\bar{G}''$	$\bar{G}''$	M_C	V_e	ξ	ρ	$(\nu)_{2,r}{}^{2/3}$	$(\nu)_{2,s}{}^{1/3}$
(kGy)	**(Pa)**	**(Pa)**	**(kg mol⁻¹)**	**(mol m⁻³)**	**(nm)**	**(kg m⁻³)**		
10	1700	300	122,18	0,85	58	1046	0,2573	0,3113
15	2876	270	68,98	1,51	46	1037	0,2591	0,2978
25	5790	117	30,82	3,36	34	1034	0,2593	0,2685

Hydrogel GI-0,25% PEO

Dose (kGy)	$\bar{G}''$ (Pa)	$\bar{G}''$ (Pa)	M_C (kg mol^{-1})	V_e (mol m^{-3})	ξ (nm)	ρ (kg m^{-3})	$(\nu)_{2,r}{}^{2/3}$	$(\nu)_{2,s}{}^{1/3}$
10	2267	332	119,64	0,86	45	1033	0,2691	0,3936
15	3452	309	73,83	1,40	38	1036	0,2671	0,3715
25	9816	207	23,73	4,36	24	1035	0,2678	0,3390

Hydrogel GI-0,1% PEO

Dose (kGy)	$\bar{G}''$ (Pa)	$\bar{G}''$ (Pa)	M_C (kg mol^{-1})	V_e (mol m^{-3})	ξ (nm)	ρ (kg m^{-3})	$(\nu)_{2,r}{}^{2/3}$	$(\nu)_{2,s}{}^{1/3}$
10	1163	206	191,74	0,53	63	1025	0,2450	0,3583
15	1716	164	127,00	0,81	53	1032	0,2460	0,3459
25	3241	82	58,25	1,77	42	1035	0,2475	0,2972

CHAPTER 9. EFFECT OF IONIZING RADIATION ON THE THERMAL STABILITY OF COLLAGEN-PVP HYDROGELS

9.1. Thermal stability of irradiated collagen

Literature data show that the collagen molecule is ten times more resistant to irradiation than other proteins (*i.e* DNA). The most important process observed in the structure of collagen under the influence of ionizing radiation (X, e-beam or γ) is the hydrolysis of the peptide bonds. If the collagen irradiation occurs in the presence of water, the destructive processes begin to predominate only at doses exceeding 50 kGy. The crosslinking of the collagen following irradiation takes place through oxidative deamination, with the formation of the iminic or aldolic bonds, conferring mechanical and thermal resistance [39]. The maximum temperature in the DSC curves reflects the denaturation temperature (T_d), and the denaturation enthalpy (ΔH_d) gives indications on intra- and intermolecular interactions [40]. Two distinct processes in the temperature range (20–400)°C [41] are indicated:

- ✓ (25–125) °C an endothermic process, attributed to thermal dehydration;
- ✓ (125–400)°C an exothermic process, attributed to thermo-oxidation decomposition.

The thermal stability of the irradiated collagen gel with doses (5 - 100) kGy was investigated by differential dynamic calorimetry in an inert nitrogen atmosphere with a heating rate of 10 K / min (figure 9.1). From the obtained DSC data it is observed that the thermal stability is different on different areas of the temperature range used. In the temperature range (20 - 80)°C an wide endothermic peak, with multiple inflections appears. It is observed that for the irradiated sample with 5 kGy, the maximum peak appears at 63.2°C, and by deconvolution it is observed that it is the sum of three independent endothermic processes, with maximums at: 47.1°C, 61.4 °C and 68.8 °C.

The temperature of 47.1°C can be attributed to the gelatinization process of collagen. With the increase of the irradiation dose up to 60 kGy, the increase of T_d and ΔH_d was observed. The conformational changes that occur in the collagen molecule after irradiation produce a decrease in the denaturation temperature. Other types of collagen molecule transformations occur through processes of gelatinization or irreversible denaturation, through which the triple-helix structure changes into random structure [42]. As several authors suggest, the endothermic peak at 61.9 ° C comes from the transition of the triple-helix structure of collagen molecules into randomly wound structures. Intra- and inter-molecular hydrogen bonds, as well as hydrogen bonds from water, give stability to the triple-helix structure of the collagen molecule.

9.2. Thermal stability of irradiated PVP

For the non-irradiated PVP solution, the dehydration process takes place at 108.6°C with an enthalpy equal to 309.1 J / g; at 5 kGy, the dehydration temperature drops to 99.5°C, and the process enthalpy increases to 674.7 J / g. In the dose range (10–50) kGy, the dehydration temperature increases from 105.2°C→115.3°C, and the enthalpy also increases from 567J/g to 620.5 J/g. The increase of the temperature required for the dehydration process about 10 degrees is due to the occurrence of intra- and intermolecular crosslinking reactions and which prevents the removal of bound water, requiring higher temperatures. The second process identified in the DSC curve appears at 444.5°C for the non-irradiated sample, and with the increase of the irradiation dose, the peak shifting is observed at slightly lower temperatures. At dose of 100 kGy, the decomposition temperature decreased to 434.5°C, indicating the existence of degradation processes of the PVP molecule. The thermal decomposition process of the irradiated and irradiated PVP takes place through two processes (figure 9.3): between (40 - 250)°C the physical loss of water occurs and between (350 - 550)°C the thermo-oxidation or decomposition processes take place. From (0-5) kGy, the maximum of the first process decreases from 105°C → 95,9 °C with a mass loss of 1.8% - 3.77%. From (0 - 50) kGy the maximum of the first process increases from 105°C→113,5°C, and the mass loss drops below 2%. At the 100 kGy dose, the maximum peak drops to 101°C and the mass loss is 3%; for the second PVP decomposition process, the corresponding peak was identified at ~ 450°C, however with the increase of the irradiation dose, no significant peak shifting was observed. From the evaluation of the results obtained above it can be concluded that both collagen and PVP do not show significant changes in terms of thermal stability when were irradiated with e-beam.

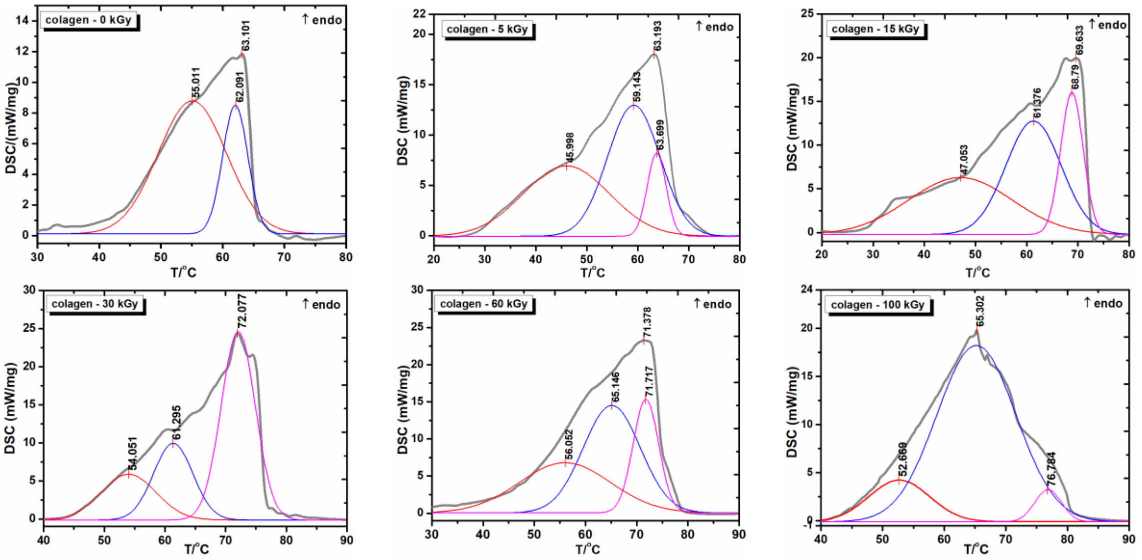


Figure 9.1. DSC curves for irradiated collagen gel

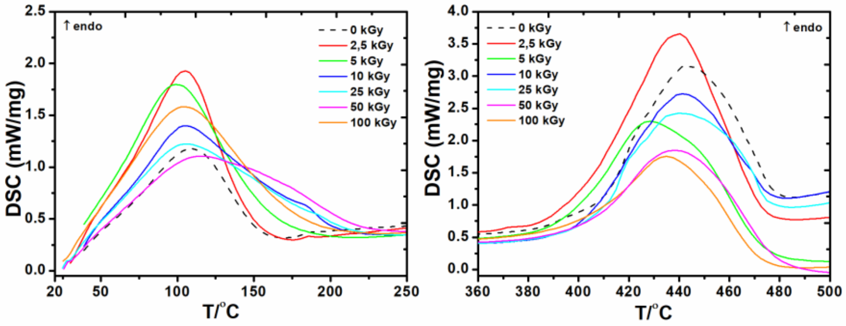


Figure 9.2. DSC curve for PVP unirradiated and irradiated (aqueous solution)

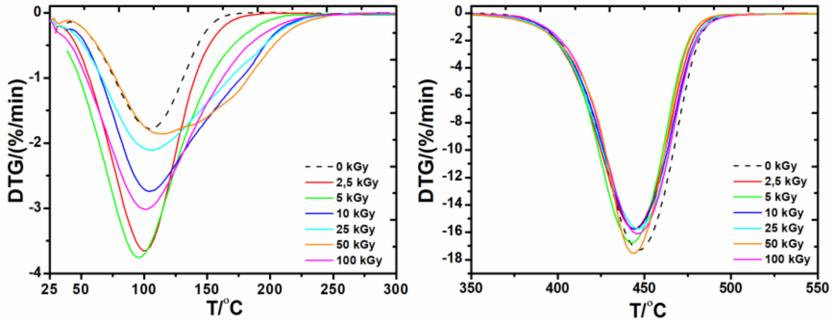


Figure 9.3. DTG curves for unirradiated and irradiated PVP

9.3. Thermal stability of collagen and PVP mixtures upon irradiation

For irradiated collagen-PVP mixtures with doses in the range (5 - 30) kGy the DSC and TG/DTG data show a glass transition characteristic to the gel. The temperature characteristic of this transition increases with the irradiation dose from 184.3°C to 204.2°C. The other peaks present in the thermal analysis curves correspond to the collagen and PVP structure in the gel structure obtained by irradiation.

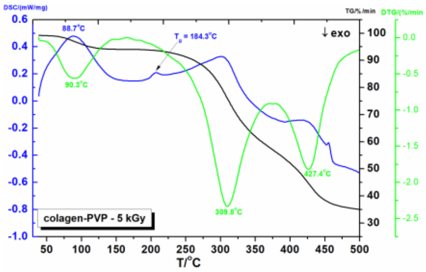


Figure 9.6. DSC, TG/DTG curves of irradiated collagen-PVP mixture with 5 kGy

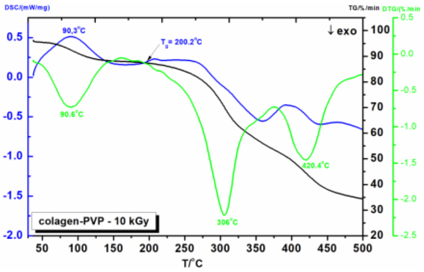


Figure 9.7. DSC and TG / DTG curves of collagen-PVP irradiated with 10 kGy

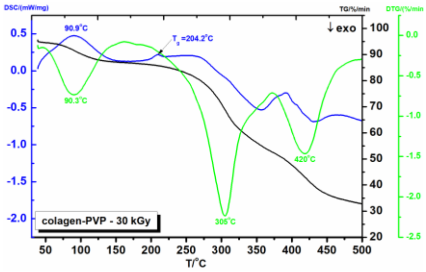


Figure 9.8. DSC and TG / DTG curves of collagen-PVP mixture irradiated with 30 kGy

GENERAL CONCLUSION

- This paper presents the experimental results regarding the synthesis and physico-chemical characterization of hydrogels obtained by cross-linking with ionizing radiation. By this method, several formulation of superabsorbent hydrogels (collagen/PVP, collagen/PVP/PEO) have been obtained, which can be the basis for obtaining dressings for the rapid healing of skin lesions. Depending on the cross-linking method, the irradiation parameters and the composition of the mixtures, hydrogels with different elastic, swelling and morphological properties were obtained, and at the same time with good quality for the intended purpose.
- • In the case of the collagen-PVP hydrogel obtained with γ-radiation in the absence of oxygen and without the addition of crosslinking agents from the analysis of the experimental results it was found that the crosslinking reaction takes place, but the properties of swelling or elasticity are reduced compared to those of hydrogels obtained by e-beam. For this system, the use of a radiation dose of 25 kGy to ensure the sterilization of the product concomitantly with the production of the cross-linking reaction, leads to a weaker hydrogel in terms of rheological properties (the elastic modulus of these gels did not exceed the value 100 Pa.)

The e-beam cross-linking of the collagen/PVP system showed obtaining of a superabsorbent hydrogels characterized by higher elasticity than those obtained with γ radiation. The addition of a small amount of water-soluble crosslinking agent, such as N'N-methylbisacrylamide (NMBA), greatly increased the swelling degree in deionised water (11 000%). Also, the stability of the hydrogels was maintained for 48 h under these conditions.

• The hydrogels obtained with e-beams, have an elastic modulus between (1800-8400) Pa, rheological parameter which is a basic feature in the evaluation of the quality of a hydrogel.

• It is worth noting that this range of elastic modulus is in good approximation corresponding to human skin or soft tissue.

• It was established that for obtaining of a superabsorbent hydrogel from collagen and PVP with stability demonstrated in physiological similar environments (pH = 7.4, 37°C, over 48 hours), with a degree of swelling over 2000%, M_C = 60 - 66 kg • mol^{-1} and ξ = 53-54 nm, this can be controlled by modifying the irradiation dose, but also by establishing adequate concentrations of the polymers which constitutes the initial solution.

• The use of increased concentrations of polymers (i.e collagen more than 1%), (PVP more than 7%) or cross-linking agents (more than 0.5%) predominates the degradation reactions leading to obtaining a hydrogel with weaker elastic properties and likely to dissociate in contact with the wounds.

• Accelerated electron beam crosslinking of collagen/PVP and collagen/PVP/PEO systems in inert atmosphere (Ar) resulted in the obtaining of hydrogels with a higher degree of cross-linking compared to those obtained in the absence of air, for which radiochemical yields cross-linking, were more than 5 times. These hydrogels had a swelling of 4000% at a dose of 25 kGy. By adding a concentration of 0.3% PEO in hydrogels composition, the degree of swelling decreased, but the G' module increased to 14000 Pa. The hydrogels obtained in an inert atmosphere were stable both in deionised water and in medium pH ranges (5.4 - 9.4). The chosen pH range ensures stability under healthy skin or infected wounds.

• Diffusion studies in deionised water and different pH media have shown that all hydrogels exhibit non-Fickian behavior, which is specific to cross-linked hydrogels. As the degree of cross-linking is higher, the diffusion decreases accordingly and occurs in two stages. The addition of PEO (non-toxic polymer) and which induces elasticity in the composition of the hydrogels improved the diffusion both in deionised water, but also in solutions with different pH.

• The M_C and ξ parameters decreased with increasing irradiation dose and the crosslinking density increased.

• The rheological characteristics of these hydrogels, similar to soft tissues, make them usable in tissue engineering. The thesis aimed at optimizing the cross-linking efficiency with the sterilization of the product, optimizing the elastic properties and stability in physiological media by adding PEO in concentrations (0.2 - 0.5)% which predominantly provides cross-linking reactions. A concentration of 0.35% PEO ensures an elasticity of up to 16,000 Pa.

• The data obtained in the thesis showed that the hydrogels can be obtained both in vacuum and in inert atmosphere, which implies different manufacturing costs, but also a different relationship between the crosslinking and degradation processes.

• It has been established that the operations: centrifugation, degassing and vacuuming before irradiation are sufficient to obtain a hydrogel with controlled properties.

• For the systems studied from the thermal point of view, in the interval of doses (5-30) kGy there are no changes compared to the non-radiated systems.

List of scientific papers published in ISI journals

1. **M. Demeter**, I.Calina, C.Vancea, M.Şen, M.G. Albu Kaya, E. Mănăilă, M. Dumitru, V. Meltzer*, E-beam processing of collagen-poly(*N*-vinyl-2-pyrrolidone) double-network superabsorbent hydrogels: structural and rheological investigation, Macromolecular Research, 27(3), 255-267, **2019. (IF$_{2018}$ = 1,758)**
2. **M. Demeter**, M. Virgolici, C. Vancea, A. Scarisoreanu, M. G. Albu Kaya, V. Meltzer*, Network structure studies on γ–irradiated collagen–PVP superabsorbent hydrogels, Radiation Physics and Chemistry, 131, 51-59, **2017. (IF$_{2017}$ = 1,435)**
3. **M. Dumitraşcu (Demeter)**, V. Meltzer, E. Sima, M. Vîrgolici, M.G. Albu, A. Ficai, V. Moise, R. Minea, C. Vancea, A. Scărişoreanu, F. Scarlat, Characterization of electron beam irradiated collagen-polyvinylpyrrolidone (PVP) and collagen-dextran (DEX) Blends, Digest Journal of Nanomaterials and Biostructures, 6(4), 1793-1803, **2011**.**(IF$_{2011}$ = 1,200)**

Other articles published in the field of doctoral thesis:

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1. **M. Dumitraşcu (Demeter)**, M.G. Albu, M. Vîrgolici, C. Vancea, V. Meltzer, Characterization of Electron Beam Irradiated Polyvinylpyrrolidone-Dextran (PVP/DEX) Blends, Solid State Phenomena, 188, 102-108, **2012**.
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