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Radiation Synthesis and Characterization of Poly(ethylene oxide)/Chitosan Hydrogels

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ABSTRACT: Recent advances in hydrogel technology have focused on finding more biocompatible, nontoxic materials intended for pharmaceutical and biomedical applications. In this study, a series of pH-sensitive hydrogels were prepared from poly(ethylene oxide) (PEO) and chitosan in aqueous solutions by electron beam irradiation. This method is a suitable tool for the formation of biocompatible hydrogels because in radiation processing no initiators or crosslinkers, potentially toxic and difficult to remove, are needed. In this frame, also the PEO and chitosan choice was based on their characteristic of low toxicity. The properties of the prepared hydrogels were investigated in terms of the gel fraction and of the swelling behavior in solutions at different pHs. Some swelling kinetic and diffusional parameters were also determined. The observed properties show that increasing the chitosan content, or lowering the pH, the crosslinking density of these networks increases inducing the formation of more stable, but less swellable, hydrogels.

KEYWORDS: poly(ethylene oxide); chitosan; hydrogel; electron beam irradiation

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INTRODUCTION

In the last years, the development and analysis of environmentally or physiologically responsive hydrogels have attracted a lot of attention. The growing interest in these "smart" hydrogels is related to their biomedical and pharmaceutical applications as they are potential candidate for carriers of bioactive macromolecules, wound dressing, and so on.[1–4] The increased demand for biocompatible, biodegradable, and nontoxic hydrogels has focused the attention on "smart" hydrogels of natural polymers, such as polysaccharides.[5–7] However, as polysaccharides dissolve easily in water, they cannot form stable hydrogels limiting their possible applications as biomedical material. Blending of natural and synthetic polymers has proved to be an effective method to overcome this limit creating new composite hydrogels with low production cost.[8–12]

In this frame, the objective of this study was to design and characterize a highly biocompatible pH-sensitive hydrogel produced by electron irradiation of aqueous solutions of poly(ethylene oxide) (PEO) and chitosan.

Chitosan [poly(1-4) 2-amino-2-deoxy-β-D-glucan] is the deacetylated derivative of chitin, which is a water-insoluble polymer found in nature, present in insect skeletons, outer shells of crabs, shrimps, lobsters, etc. and fungal cell walls.[13] Chitosan has a low toxicity, possesses antibacterial properties, is biodegradable, and biocompatible. The presence of amino groups makes it soluble in dilute acidic solutions where it is positively charged because of its pK value $\sim$ 6.5.[6,13] Being the only positively charged polysaccharide, chitosan can form electrostatic complexes with negatively charged species such as proteins, anionic polyelectrolytes, drugs, and low-molecular-weight anions.[14,15] Such a characteristic has been attributed to the electrostatic attractive force between the positive charges of the amine groups along chitosan chains and the negative charges of the phospholipid structure of the cell membranes.[16,17] Because of the poor mechanical properties of chitosan upon its application, pharmaceutical formulations based on chitosan (such as films, beads, and microspheres) have been prepared by chemical crosslinking in the presence of other hydrophilic polymers and particularly in the presence of PEO.[18–27]

PEO is the simplest water-soluble polymer. Its chemical structure—(CH_2CH_2O)—contains the just right balance between hydrophobic and hydrophilic interactions to make it soluble in water for a wide range of temperatures and concentrations. In particular, in water solution, PEO acts as a proton acceptor, whereas chitosan acts as a proton donor, giving rise to a homogeneous mixture with peculiar biological characteristic. For

example, the presence of PEO increases the permeability and blood compatibility of chitosan.[23,28,29] The platelet adhesion and activation are also significantly reduced on chitosan PEO membranes.[23] As it is widely reported in literature, chitosan/PEO blends are often chemically crosslinked with glutaraldehyde, formaldehyde, epoxy, or genipin,[22–26] but, to the best of our knowledge, no research exists on PEO/chitosan hydrogels formed by electron beam (EB) irradiation.

EB irradiation is considered one of the most convenient and effective methods for the formation of hydrogels for biomedical application, because in radiation processing no initiators or crosslinkers, potentially toxic and difficult to remove, are needed. It also offers the advantages of combining hydrogel formation and sterilization in one step. Moreover, the degree of crosslinking, which strongly affects the properties of the hydrogel, can be easily controlled by varying the adsorbed dose.

Notwithstanding EB irradiation has been widely applied up to now to prepare hydrogels from PEO[30,31] as well as from natural polymers,[32–34] and there is a lack of information on preparation of hydrogels by EB irradiation starting from a PEO/chitosan water solution.

In this work, a series of PEO and PEO/chitosan hydrogels were prepared by EB irradiation. As a preliminary phase, the swelling and stability properties of the prepared pH-sensitive hydrogels were investigated. In particular, some swelling kinetics and diffusional parameters were determined by dynamic swelling studies.

EXPERIMENTAL

Materials and Preparation of the Hydrogels

PEO, M_W 1 × 10^5, and low-molecular-weight chitosan were purchased from Aldrich (Milwaukee, WI). Chitosan has a degree of deacetylation between 75 and 85% according to the supplier.

Chitosan was dissolved in 1% aqueous acetic acid at room temperature and left overnight with continuous mechanical stirring to obtain a 2 wt % solution. A 5 wt % solution of PEO in water was prepared by dissolving PEO in double distilled deionized water at room temperature with a stirrer. The solutions were filtered to remove any undissolved matter. The mixed solutions were prepared adding to the PEO solution 10, 5, and 1 wt % of the chitosan solution; the chitosan/PEO weight ratios are 4, 2, and 0.4%, respectively. These samples will be denominated in the text as PEO-CH 10%, PEO-CH 5%, and PEO-CH 1%, respectively.

Then, the solutions were stirred for 8 h to obtain homogeneous solutions. To remove bubbles, the solutions were placed in an ultrasonic water bath for 15 min. The prepared solutions were left to rest for about 4 h at room temperature; then, they were poured into polystyrene cuvette and irradiated. After irradiation, the crosslinked samples were cut into disks, weighed (W_r), and freeze dried until a constant weight (W_d) was reached. The PEO disks were weighted both in air and heptane to evaluate the volume of the gel in the relaxed and dried state.

Irradiation

Radiation-induced crosslinking of PEO and PEO/chitosan hydrogels was performed by means of the 5 MeV electron linac of the Physics Department, Messina University.[35] The linac has been designed and setup to develop new applications of radiation processing; in particular, a pulsed EB as that delivered by this accelerator allows to vary dose rate among wide ranges, thus optimizing radiation processing treatments in view of their industrial application. For example, in the framework of hydrogel crosslinking, the use of intense pulses of radiation can promote intramolecular crosslinking, thus inducing the formation of nanogels.[36]

The 1 kW, 5 MeV electron linac used for this work was properly designed to obtain an autofocusing structure, allowing to avoid use of focusing magnets, thus noticeably minimizing the accelerator dimensions. As one can easily see, the wide range of variability of its main operational parameters allows us to modify dose rate by acting on the peak current, on the pulse repetition rate, and on the sample position. In this way, a great number of experimental conditions can be achieved, thus optimizing each treatment in view of its possible industrial application. Radio frequency power is supplied to the accelerating structure by a magnetron generator, for which a properly designed pulse-forming circuit has been developed, charged through an inductance, and triggered by an hydrogen-filled thyratron, delivering to the magnetron a 45 kV, 90 A pulse. The electron injector consists of a rhenium oxide-emitting cathode, supplied by a compact pulse-forming circuit, providing a 13 kV, 10 A pulse.

As a first screening, PEO hydrogels were irradiated at a constant dose of 50 kGy, but with a variable dose rate, ranging from 2 to 32 Gy/s. These dose rates were obtained by maintaining the repetition rate constant at 1.5 Hz and by varying the peak current between 1 and 40 mA. A particular value of 4 Gy/s was chosen as the optimal dose rate, and for this reason, subsequent irradiations of PEO/chitosan samples were made at this value.

Gel Content

To evaluate the gel content, a dried disk was immersed in hot distilled water at 45°C for 72 h, with water being changed every 5 h. The insoluble part of the hydrogel made up of only the crosslinked hydrogel was freeze dried to a constant weight (W_g). The gel fraction was evaluated as follows:

$$\text{Gel fraction}(\%) = W_g/W_d \times 100, \quad (1)$$

where W_d is the initial weight of the dried gel.

Swelling Behavior

The dried hydrogels were immersed in 50 mL of swelling medium at 37°C. Swelling studies were carried out in media of different pHs ranging between 1.2 and 7.5 prepared from the dilution of stock solutions of HCl and NaOH using deionized distilled water. The ionic strength of the media was adjusted to 0.1M by the addition of NaCl. The pH values were confirmed by a pH meter.

At regular intervals, the hydrogel disks were removed from the medium and weighted after removing surface-absorbed water with filter paper. Then, the samples were immediately replaced in the same swelling medium. The samples were swollen for 72 h until an equilibrium state of swelling was achieved. The water

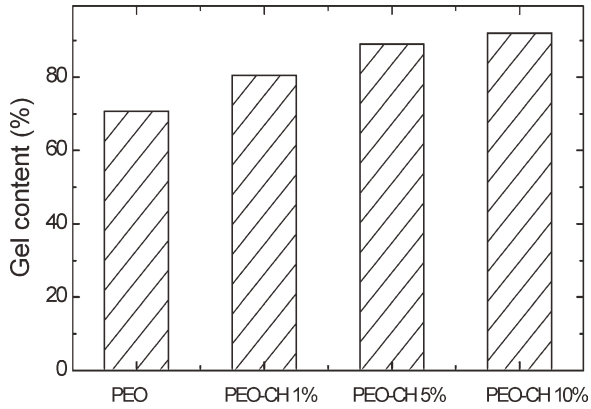

Figure 1. Comparison of the gel content of the investigated hydrogels.

contents at time t of the swollen hydrogels were calculated by using the following relation:

$$S(\%) = \frac{(W_s - W_d)}{W_d} \times 100, \quad (2)$$

where W_s is the weight of the swollen gel at time t. Each swelling experiments was repeated three times, and the average values are reported.

The equilibrium degree of swelling (EDS) of the gel was calculated as follows:

$$\mathrm{EDS} = \frac{(W_e - W_d)}{W_d} \times 100, \quad (3)$$

where W_e is the weight of the gel at the equilibrium. The weights of the PEO samples in the relaxed, dry, and equilibrium swollen state were also measured in heptane using a hanging basket apparatus.

RESULTS AND DISCUSSION

Gel Fraction

Previous studies on irradiation of PEO aqueous solution have shown that free radical crosslinking predominates over scission.[37,38] The dose rate and total dose used in this work are the minimum irradiation conditions at which gel formation is observed starting from a 5 wt % PEO/water solution. The same irradiation conditions are not sufficient to assure the crosslinking of the 1 wt % chitosan aqueous solution. In fact, the crosslinking of natural polymers can be achieved only in very concentrated aqueous solutions.[32] The gel content is an important parameter especially for biomedical applications where it is required a high stability of hydrogels during fabrication and utilization. Applying eq. (1) it was observed that addition of chitosan from 1, 5, and 10% resulted in an increase of the gel fraction up to 96%, see Figure 1. As reported above, this could be due to the formation of an interpenetrating network characterized by a crosslinking density that increases as the chitosan content increases so inducing the formation of more stable hydrogels.

Swelling Behavior

The swelling properties are influenced by physical factors, such as the crosslinking density, and by the environmental conditions. In this work, we studied the pH sensitivity of the prepared hydrogels in a pH range between 1.2 and 7.5. Figure 2 shows, as an example, the swelling curves for PEO and the PEO/chitosan hydrogels at different pH values.

As shown, swelling increases with time, reaching a limiting value in about 24 h for all the samples, being maximum for hydrogel without chitosan, and decreases when increasing the chitosan content. Therefore, the presence of chitosan enhances the stability of the gel but lowers the swelling ratio because of the tighter structure induced by the higher crosslinking.

With reference to the pH dependence, it can be observed that although pure PEO does not show any pH sensitivity, EDS(%)

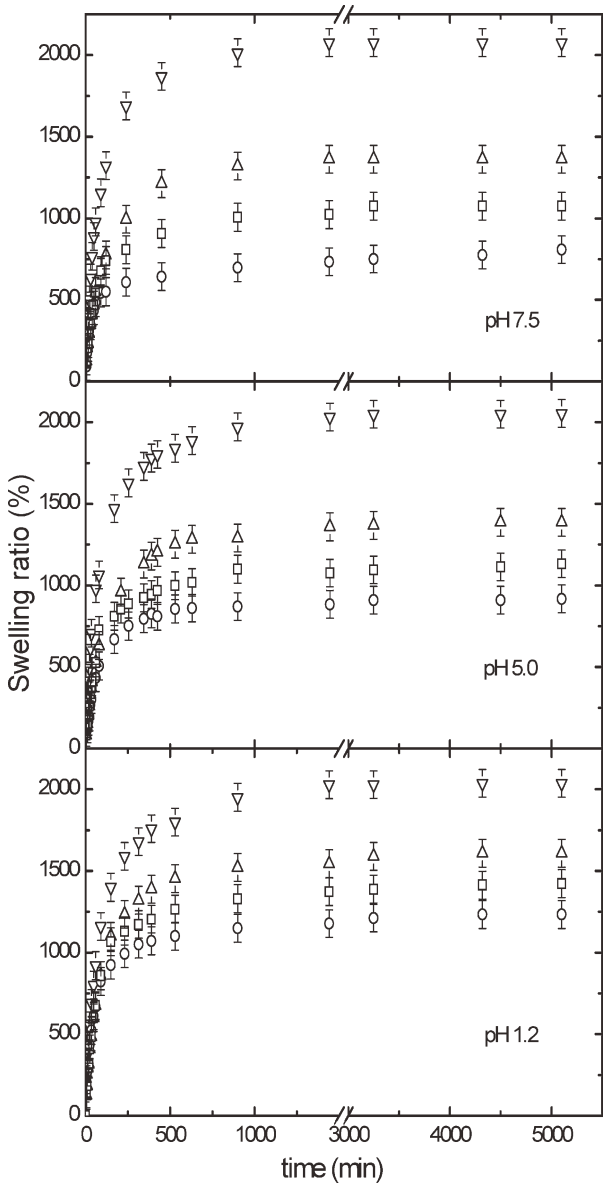

Figure 2. Swelling behavior of PEO and PEO/chitosan hydrogels at different pH values with various concentration of chitosan: PEO (down triangles), PEO-CH 1% (up triangles), PEO-CH 5% (squares), and PEO-CH 10% (circles).

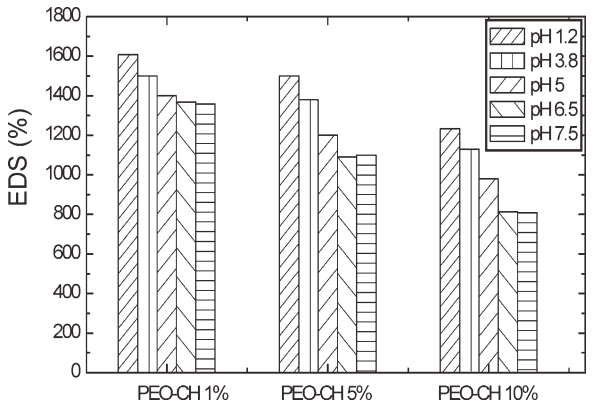

Figure 3. Equilibrium swelling degree of PEO/chitosan hydrogels at different pH values.

$\cong$ 2050, hydrogels with more chitosan show a larger pH dependence of the swelling capacity. In particular, according to the cationic nature of the PEO/chitosan hydrogels, the EDS decreases by increasing the pH of the swelling medium, see vFigure 3. The increased swelling in acidic condition may be ascribed to the presence of more amino groups that can be protonated. This protonation induces electrostatic repulsions between the polymer segments and hence a greater swelling of the hydrogels. As the pH increases, amino groups become deprotonated and the repulsion receded.

A last comment about the swelling property refers to the rate of swelling that is particularly high for freeze-dried hydrogels. Before giving an evaluation of this rate according to a physical model, we can observe that the swelling of the PEO/chitosan hydrogels reaches 80% in acidic conditions in less than 4 h. This property is very advantageous in designing a hydrogel for drug delivery in the gastrointestinal tract, because a fast swelling enables a rapid diffusion and delivering of the drug contained in the hydrogel.

Determination of the Network Parameter M_c

As it is widely reported in literature, the equilibrium swelling data can be used to evaluate the network property of the polymeric hydrogels. In particular, the structure of hydrogels that do not contain ionic moieties can be analyzed using the Peppas–Merrill equation, a modification of the Flory–Rehner equation that takes into account the presence of a solvent during the gel preparation.[39,40] Through this equation, one can evaluate an average value for the molecular weight of the polymer chain between two consecutive crosslinking points ($\overline{M}_c$) and the corresponding mesh size ξ, also referred to as the pore size. According to the equilibrium swelling theory, first it is necessary to calculate the polymer volume fraction in the swollen, v_{2s}, and relaxed state, v_{2r}, that is, after irradiation. For this, the following equation can be used[41]:

$$v_{2,x} = \frac{W_{a,d} - W_{h,d}}{W_{ax} - W_{hx}}. \tag{4}$$

The subscript x can indicate the swollen state, represented by the subscript s, or the relaxed state, represented by the subscript r. The subscripts a and h are indicative of weighing in air and heptane. The subscript d indicates the dried state. Once defined v_{2s} and v_{2r}, one can calculate $\overline{M}_c$ through the following Peppas–Merrill equation:

$$\frac{1}{\overline{M}_c} = \frac{2}{\overline{M}_n} - \frac{(\overline{v}/V_1)\left[\ln(1 - v_{2,s}) + v_{2,s} + \chi v_{2,s}^2\right]}{v_{2,r}\left[(v_{2,s}/v_{2,r})^{1/3} - (1/2)(v_{2,s}/v_{2,r})\right]}. \tag{5}$$

In this equation, $\overline{M}_n$ is the average molecular weight number of the linear polymer before crosslinking. V_1 is the molar volume of water (18.1 cm^3/mol) and $\overline{v}$ is the specific volume of the polymer calculated by equation

$$\overline{v} = \frac{1}{\rho_h}\frac{W_{a,d} - W_{h,d}}{W_{a,d}}, \tag{6}$$

where ρ_h is the density of heptane (0.684 g/cm^3). Finally, χ is the Flory–Huggins interaction parameter. The main drawback of this approach is that the χ parameter must be determined very accurately. Although for the PEO/water solution it is known quite precisely ($\chi = 0.45$), up to now there is not an exact evaluation of this parameter for the PEO/chitosan solvent.

The procedure suggested by Li et al.[42] overcomes this problem. In fact, the Flory–Huggins interaction parameter χ can be evaluated directly thorough the swelling measurements of the hydrogel into a mixture of water and methanol. In more details, according to the Flory–Huggins theory, M_c can be expressed as the following equation:

$$M_c = S^{5/3}\rho V_1/(0.5 - \chi), \tag{7}$$

where S represents the swelling capability of the hydrogel, $S = (W_s - W_d)/W_d$; ρ denotes the density of hydrogel evaluated with the weighing bottle method; V_1 denotes the molar volume of the solvent used for swelling studies; and χ is the just cited Flory–Huggins parameter. To determine the χ value, the linear relationship between χ and C (the volume fractions of methanol in methanol/water mixture) was established[42,43]

$$\chi = K_1 C + K_2. \tag{8}$$

Thus, if $K_2 < 0.5$, the following equation can be obtained:

$$\rho V_1 S^{5/3}_{\text{methanol}-\text{water}} = M_c(0.5 - K_1 C). \tag{9}$$

The plots of $\rho V_1 S^{5/3}_{\text{methanol}-\text{water}}$ against C give straight line, and the M_c can be calculated according to the slope of the resulting lines.[42,43]

In this work, both these approaches were used to evaluate the network parameter M_c for the PEO hydrogel. With the Peppas–Merrill equation a value of $M_c \approx 3790$ is obtained in good agreement, within the experimental error, with the value of $M_c \approx 3680$ obtained by using the second approach. Once the goodness of these approaches was proved, the M_c values for the PEO/chitosan hydrogels were determined by following the second approach. In such a case, the obtained values for the interjunction molecular weight are as follows: $M_c \approx 2980$ for PEO-CH 1%, $M_c \approx 1950$ for PEO-CH 5%, and $M_c \approx 1120$ for PEO-CH 10%.

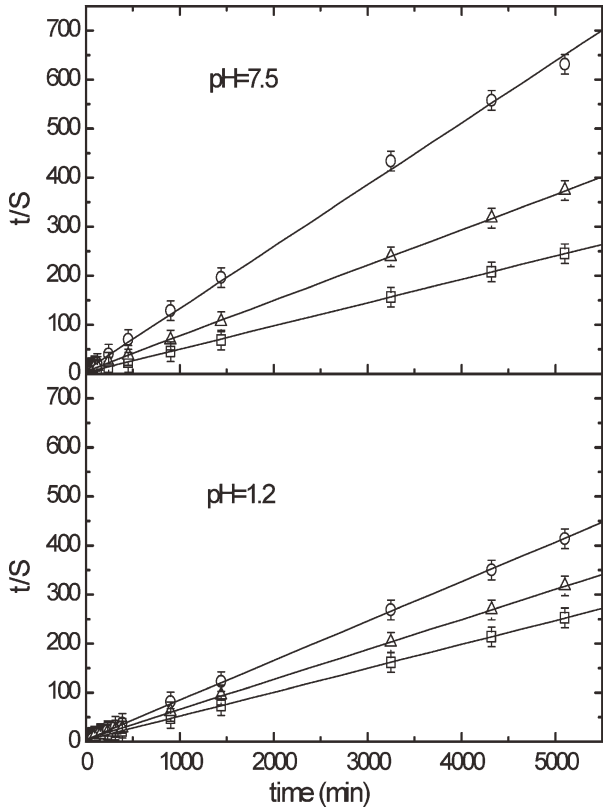

Figure 4. Swelling rate curves of PEO (triangles), PEO-CH 1% (squares), and PEO-CH 10% (circles) at pH values 7.5 and 1.2.

Finally, from the interjunction molecular weight, M_c, an approximate value of the mesh size ξ of the PEO hydrogel network was calculated by the equation:

$$\xi = v_{2,s}^{-1/3}\left(\frac{2C_n\overline{M}_c}{M_r}\right)^{1/2} l. \tag{10}$$

Here, M_r is the molecular weight of the repeating unit (44), C_n is the characteristic ratio of the polymer (3.8), and l is the bond length along the polymer chain (1.54 Å). From the M_c value obtained for the PEO hydrogel, it was possible to evaluate a value for the mesh size ξ of about 110 Å. Even if we cannot apply the same procedure to evaluate the PEO/chitosan hydrogel mesh size, we can, however, state, with a certain reliability, that the pore sizes for these networks are smaller than 10 nm. In fact, as just pointed out, the reduced swelling, accompanied by an increased gel content and a decreased interjunction molecular weight observed for PEO/chitosan in comparison to PEO hydrogel, is related to an increase in crosslinking density and hence to a reduction in mesh size for these hydrogels.

Swelling Kinetics and Diffusion

To examine the swelling kinetics, several models have been proposed. According to Katime et al.,[44] the swelling dynamics of the hydrogels can be described by the relation:

$$\frac{t}{S} = \frac{1}{k_s S_{\max}^2} + \frac{1}{S_{\max}} t, \tag{11}$$

where k represents second-order swelling kinetics, $S_{\max}$ is the maximum swelling, and S is the swelling at time t. To test this model, t/S vs. t graphs are plotted and a representative graph is reported in Figure 4 for PEO, PEO-CH 1% and PEO-CH 10% hydrogels at pH 1.2 and 7.5, respectively. The linear regressions of each swelling curve at all the pHs investigated indicate that they all obey second-order kinetics during the time of observation. From the slope and intersections of the lines, the maximum swelling, $S_{\max}$, and the swelling rate constant, k_s, are calculated and reported in Table I. As one can see, the results of the kinetic model are in agreement with swelling experiments; it is also interesting to observe that with respect to k_s, at each pH value, it increases by increasing the chitosan content, because the amount of water absorbed is lower and the equilibrium swelling is attained faster.

Exploiting the swelling experiments, the nature of water diffusion into our hydrogels can also be determined. To this purpose, the initial swelling data were fitted by using the following empirical expression[45,46]:

$$\frac{M_t}{M_\infty} = F = kt^n, \tag{12}$$

where M_t is the amount of water absorbed at time t, M_∞ is the amount of water absorbed at equilibrium, k is the empirical rate constant, and n is the diffusion exponent indicative of the transport mechanism.

As the solvent penetrates into the polymer, the polymer network rearranges itself reaching an equilibrium when the elastic restoring force of the network balances the osmotic pressure driving the solvent into the polymer. The viscoelastic properties of the polymer play a very important role in this process as they control the polymer response to the change in configuration. Based on the rate of diffusion relative to the polymer relaxation rate, one can distinguish three classes of diffusion[47]:

1. Case I or Fickian diffusion ($n = 0.5$) in which the relaxation coefficient is negligible during transient sorption;
2. Case II ($n = 1$) or relaxation-balanced diffusion in which morphological changes are abrupt;
3. Non-Fickian or anomalous diffusion ($0.5 < n < 1$), which occurs when the diffusion and relaxation rates are comparable. A value of $n < 0.5$ indicates a pseudo-Fickian behavior of diffusion but with a slower approach to final equilibrium.

For all the investigated hydrogels, ln F vs. ln t graphs were plotted and representative results are shown in Figure 5 at two different pH values. From the slope and intercept of the curves in the first 60% of water uptake, the n and k parameters were calculated and the obtained values are reported in Table I. These parameters are related to the diffusion coefficient D through the relation:

$$D^n = \frac{k(\pi r^2)^n}{4}, \tag{13}$$

where D is in cm^2/s, t in s, and r is the radius of the dry gel. The diffusion coefficients of water moving through the prepared hydrogels are also reported in Table I.

As it can be observed, the diffusion of water into hydrogels is found to have a Fickian character only for PEO and PEO/chitosan hydrogels at the lowest chitosan concentration. The

Table I. Swelling and Diffusion Parameters of PEO and PEO/Chitosan Hydrogels at Various pH Values

	S_{max} (%)	k_s (10^4)	n	k (10^2)	$D \times 10^5$ (cm^2/s)
PEO	22.6	7.64	0.51	5.7	1.69
pH 1.2					
PEO-CH 1%	16.39	7.93	0.50	5.5	1.33
PEO-CH 5%	14.20	10.15	0.46	7.6	1.28
PEO-CH 10%	12.33	14.88	0.42	10.6	1.24
pH 3.8					
PEO-CH 1%	15.00	8.1	0.49	5.7	1.20
PEO-CH 5%	13.80	10.6	0.45	7.7	1.08
PEO-CH 10%	11.30	15.8	0.41	10.5	0.98
pH 5					
PEO-CH 1%	14.20	9.0	0.48	6.0	1.12
PEO-CH 5%	12.10	11.8	0.44	8.2	1.02
PEO-CH 10%	9.80	19.2	0.4	11.8	1.05
pH 6.5					
PEO-CH 1%	13.68	9.3	0.48	6.0	1.12
PEO-CH 5%	10.90	12.2	0.44	8.4	1.08
PEO-CH 10%	8.13	19.9	0.39	12.0	0.87
pH 7.5					
PEO-CH 1%	13.88	9.38	0.47	6.5	1.10
PEO-CH 5%	11.00	12.43	0.43	8.8	9.86
PEO-CH 10%	8.08	20.42	0.39	12.1	0.89

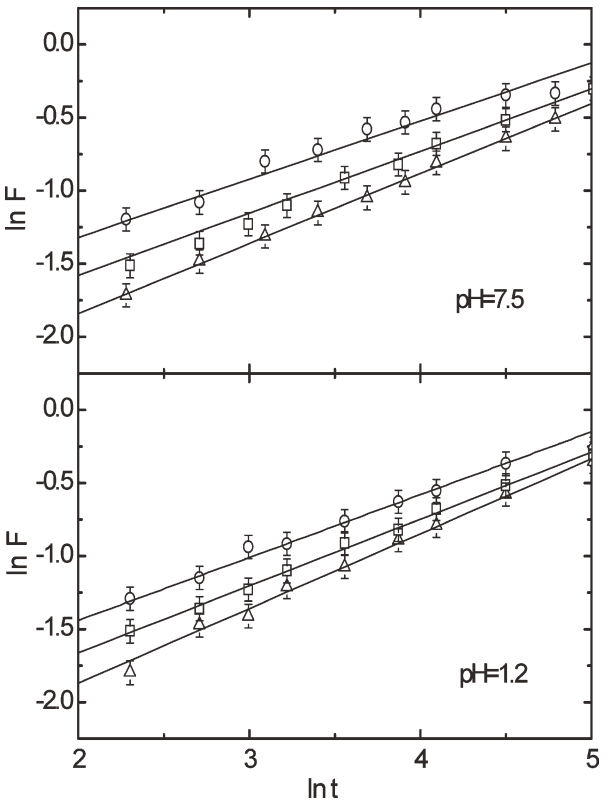

Figure 5. Plot of ln F vs. ln t for PEO (triangles), PEO-CH 1% (squares), and PEO-CH 10% (circles) at pH values 7.5 and 1.2.

hydrogels with a higher chitosan content show a pseudo-Fickian behavior. This might be attributed both to the higher inhomogeneity and to the denser network structure of these hydrogels, which hindered the diffusion of water and thus prolonged the final equilibrium process. The decreasing of the n parameter with increasing the pH values confirms that the restricted diffusion of water is ruled by the tighter structure of the hydrogels. The crosslink density directly affects the mechanical deformation of the hydrogels. In fact, the interior network structure of the hydrogels becomes more porous with the decrease of the crosslinking level, which provides numerous water channels for the diffusion of water. This is also supported by the values of the diffusion coefficients determined for our hydrogels. In fact, one can observe a slowing down of the diffusion of water as the swelling properties of the gels diminish. This is obviously due to the confined motion of the water molecules inside smaller and smaller channels. The diffusion of water inside the PEO hydrogel with the largest pore size (110 Å) is 1.69×10^{-5} cm^2/s (slower than that of water in bulk H_2O, about 2.2×10^{-5} cm^2/s at room temperature) and lowers to 0.87×10^{-5} cm^2/s for the PEO/chitosan hydrogels at the highest concentration and pH values. It is interesting to observe that this value for the diffusion is comparable with that reported in a recent work[48] for the diffusion coefficient of water at room temperature inside the gels with a pore size between 30 and 50 Å.

CONCLUSIONS

In this study, a series of pH-dependent hydrogels were prepared from aqueous solutions of PEO and chitosan by EB irradiation.

The wide range of variability of main operational parameters of the pulsed 5 MeV electron linac allows us to test and optimize different experimental conditions for the preparation of the hydrogels. Irradiation at a dose rate of 4 Gy/s showed a remarkable growth of the gel fraction up to 96% by increasing chitosan from 1 to 10% and correspondingly a drastic decrease of the swelling ratio. Because of the cationic nature of chitosan, the hydrogels were also found to be sensitive to pH, showing an increased swelling in acidic condition. Increasing the chitosan content, the crosslinking density of these networks increases, thus inducing the formation of more stable, but less swellable, hydrogels.

REFERENCES

1. Petka, W. A.; Harden, J. L.; McGrath, K. P.; Wirtz, D.; Tirrell, D. A. *Science* **1998,** *281*, 389.
2. Qui, Y.; Park, K. N. *Adv. Drug Delivery Rev.* **2001,** *53*, 321.
3. Kim, B.; Peppas, N. A. *J. Biomater. Sci. Polym. Ed.* **2002,** *13*, 1271.
4. Peppas, N. A.; Hilt, J. Z.; Khademhosseini, A.; Langer, R. *Adv. Mater.* **2006,** *18*, 1345.
5. Chen, J.; Jo, S.; Park, K. *Carbohydr. Polym.* **1995,** *28*, 69.
6. Francis, S. J. K.; Matthew, H. W. T. *Biomaterials* **2000,** *21*, 2589.
7. Cascone, M. G.; Barbani, N.; Cristallini, C.; Giusti, P.; Ciardelli, G.; Lazzeri, L. *J. Biomater. Sci. Polym. Ed.* **2001,** *12*, 267.
8. Stevens, M. P. Polymer Chemistry—An Introduction; Oxford University Press: Oxford, UK, **1999.**
9. Zhai, M.; Yoshii, F.; Kume, T.; Hashim, K. *Carbohydr. Polym.* **2002,** *50*, 295.
10. Kim, G.; Choi, Y.; Kim, M.; Park, Y.; Lee, K.; Kim, I.; Hwang, S.; Noh, I. *Curr. Appl. Phys.* **2007,** *7S1*, e28.
11. Li, J.; Zivanovic, P. M.; Davidson, P. M.; Kit, K. *Carbohydr. Polym.* **2010,** *79*, 786.
12. Bhattarai, N.; Gunn, J.; Zhang, M. *Adv. Drug Delivery Rev.* **2010,** *62*, 83.
13. Muzzarelli, R. A. A. Chitosan in Natural Chelating Polymers; Pergamon Press: London, UK, **1973.**
14. Kim, T. H.; Jiang, H. L.; Jere, D.; Park, I. K.; Cho, M. H.; Nah, J. W.; Choi, Y. J.; Akaike, T.; Cho, C. S. *Prog. Polym. Sci.* **2007,** *32*, 726.
15. Jayakumar, R.; Chennazhi, K. P.; Muzzarelli, R. A. A.; Tamura, H.; Nair, S. V.; Selvamurugan, N. *Carbohydr. Polym.* **2010,** *79*, 1.
16. Dillon, G. P.; Yu, X. J.; Bellamkonda, R. V. *J. Biomed. Mater. Res.* **2000,** *51*, 510.
17. Prasitsilp, M.; Jenwithisuk, R.; Kongsuwan, K.; Damrongchai, N.; Watts, P. *J. Mater. Sci.: Mater. Med.* **2000,** *11*, 773.
18. Zhang, Q.; Liu, L.; Ren, L.; Wang, F. *J. Appl. Polym. Sci.* **1997,** *64*, 2127.
19. Yao, K. D.; Peng, T.; Goosen, F. A.; Min, J. M.; He, Y. Y. *J. Appl. Polym. Sci.* **1993,** *48*, 343.
20. Gong, P.; Zhang, L.; Zhuang, L.; Lu, J. *J. Appl. Polym. Sci.* **1998,** *68*, 1321.
21. Neto, C. G. T.; Giacometti, J. A.; Job, A. E.; Ferreira, F. C.; Fonseca, J. L. C.; Pereira, M. R. *Carbohydr. Polym.* **2005,** *62*, 97.
22. Khalid, M. N.; Agnely, F.; Yagoubi, N.; Grossiord, J. L.; Courraze, G. *Eur. J. Pharm. Sci.* **2002,** *15*, 425.
23. Amiji, M. M. *Biomaterials* **1995,** *16*, 593.
24. Song, J. J. M. *J. Appl. Polym. Sci.* **2006,** *102*, 436.
25. Neto, C. G. T.; Dantas, T. N. C.; Fonseca, J. L. C.; Pereira, M. R. *Carbohydr. Res.* **2005,** *340*, 2630.
26. Nasir, N. F. M.; Zain, N. M.; Raha, M. G.; Kadri, N. A. *Am. J. Appl. Sci.* **2005,** *2*, 1578.
27. Song, J. J. M.; Hourston, D. J. *Biomacromolecules* **2004,** *5*, 162.
28. Zhang, M.; Li, X. H.; Gong, Y. D.; Zhao, N. M.; Zhang, X. F. *Biomaterials* **2002,** *23*, 2641.
29. Anderson, D.; Nguyen, T.; Lai, P. K.; Amiji, M. *J. Appl. Polym. Sci.* **2001,** *80*, 1274.
30. Rosiak, J. M. Radiation Effects on Polymers; American Chemical Society: Washington, DC, **1991.**
31. Branca, C.; Magazù, S.; Maisano, G.; Auditore, L.; Barnà, R. C.; De Pasquale, D.; Emanuele, U.; Trifirò, A.; Trimarchi, M. *J. Appl. Polym. Sci.* **2006,** *102*, 820.
32. Zhao, L.; Mitomo, H.; Nagasawa, N.; Yoshii, F.; Kume, T. *Carbohydr. Polym.* **2003,** *51*, 169.
33. Fei, B.; Wach, R. A.; Mitomo, H.; Yoshii, F.; Kume, T. *J. Appl. Polym. Sci.* **2000,** *78*, 278.
34. Wach, R. A.; Mitomo, H.; Yoshii, F.; Kume, T. *J. Appl. Polym. Sci.* **2001,** *81*, 3030.
35. Auditore, L.; Barnà, R. C.; De Pasquale, D.; Italiano, A.; Trifirò, A.; Trimarchi, M. *Phys. Rev. ST-AB* **2004,** *7*, 030101.
36. Rosiak, J. M.; Ulanski, P.; Kadlubowski, S. IAEA-TECDOC-1438; IAEA: Bologna, Italy, **2005.**
37. Grollman, U.; Schnabel, W. *Makromol. Chem.* **1980,** *181*, 1215.
38. Matheson, M. S.; Mamou, A.; Silverman, J.; Rabani, J. *J. Phys. Chem.* **1973,** *77*, 2420.
39. Peppas, N. A.; Merrill, E. W. *J. Polym. Sci. Polym. Chem. Ed.* **1976,** *14*, 441.
40. Barr-Howell, B. D.; Peppas, N. A. *Polym. Bull.* **1985,** *13*, 91.
41. Carr, D. A.; Peppas, N. A. *Macromol. Biosci.* **2009,** *9*, 497.
42. Li, X.; Xu, S. M.; Wang, J.; Chen, X.; Feng, S. *Carbohydr. Polym.* **2009,** *75*, 688.
43. Wang, W.; Wang, Q.; Wang, A. *Macromol. Res.* **2011,** *19*, 57.
44. Katime, I.; Valderutten, N.; Quintana, J. R. *Polym. Int.* **2001,** *50*, 869.
45. am Ende, M. T.; Peppas, N. A. *J. Controlled Release* **1997,** *48*, 47.
46. Peppas, N. A.; Franson, N. M. *J. Polym. Sci. Polym. Phys. Ed.* **1983,** *21*, 983.
47. Alfrey, T.; Gurnee, E. F.; Lloyd, W. G. *J. Polym. Sci. Part C* **1996,** *12*, 249.
48. Ghugare, S. V.; Chiessi, E.; Telling, M. T. F.; Deriu, A.; Gerelli, Y.; Wuttke, J.; Paradossi, G. *J. Phys. Chem. B* **2010,** *114*, 10285.