The swelling behavior of chitosan hydrogels membranes obtained by UV- and γ-radiation

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Abstract

In order to develop a non toxicity and biocompatible hydrogel system with potential ability in biotechnology based on membranes of polyethylene glycol (PEG 300 and 400) and chitosan were prepared under ultraviolet light (UV) and γ-radiation curing, at room temperature. The hydrogels were characterized by FTIR spectroscopic, UV absorption spectroscopy, thermal analysis and scanning electronic microscopy. The swelling behaviors were investigated in terms of time of swelling, irradiation time, and pH. The hydrogel with PEG 300 showed unusual swelling behavior.
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1. Introduction

In recent years, biodegradable polymeric systems have gained importance for design of surgical devices, artificial organs, drug delivery systems with different routes of administration, carriers of immobilized enzymes and cells, biosensors, ocular inserts, and materials for orthopedic applications [1]. These polymers are classified as either synthetic (polyesters, polyamides, polyanhydrides) or natural (polyamine acids, polysaccharides) [2]. Polysaccharide-based polymers represent a major class of biomaterials, which includes agarose, alginate, carageenan, dextran and chitosan [3,4].

Chitosan, β(1,4)2-amino-2-D-glucose, is a cationic biopolymer produced by alkaline *N*-deacetylation of chitin, which is the main component of the exoskeleton of crustaceans of crab, shrimp and krill [5]. This polycationic biopolymer is generally obtained by alkaline deacetylation of chitin, which is the main component of shrimps. The main parameters influencing the characteristics of chitosan are its molecular weight (M_w) and its degree of deacetylation (DD), representing the proportion of deacetylated units [6]. Chitosan is currently receiving a great deal of attention for medical and pharmaceutical applications. The main reasons for this increasing interest are undoubtedly due to its attractive intrinsic properties. Indeed, chitosan is known for its biocompatibility allowing its use in various medical applications such as topical ocular application [7], implantation [8] or injection [9]. Moreover, chitosan is metabolized by certain human enzymes, e.g. lysozyme, and can be considered as biodegradable [10,11] which has opened up avenues for modulating drug release in vivo in the treatment of various diseases. These chitosan-based delivery systems range from microparticles to nanoparticles [12], to gels [13] and films [14]. In addition, it has been reported that chitosan acts as a penetration enhancer by opening epithelial tight-junctions [15,16]. Due to its positive charges at physiological pH, chitosan is also bioadhesive, which increases retention at the site of application [17,18]. Chitosan also promotes wound-healing [19,20] and has bacteriostatic effects [21,22]. Finally, chitin is abundant in nature and its derivate products has low cost

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and ecological interesting [23] with commercial alternatives in north east of Brazil.

On the other hand, photocrosslinkable polymers could provide a clean and convenient biomedical-related application. Photopolymerization is a process that uses light to convert a liquid macromer/prepolymer to a solid gel. With the control of photoinitiating conditions, this process can be carried out under mild and cytocompatible conditions [24]. Nevertheless, several synthetic polymers, due to unreacted toxic monomers remained during polymerization procedures, might contain carcinogenic or toxic substances. Hence, the use of natural biodegradable materials has recently received lots of attention [25–28].

Many reports have discussed the safety of its photoreactive derivatives in medical uses such as wound dressing, biological adhesive and drug delivery of photocrosslinkable chitosan [29–31].

Furthermore, chitosan and its derivatives have low water solubility attributed to their rigid structure and crystallinity. To enhance the solubility in water, chitosan is modified by chemical reaction or copolymerized with hydrophilic polymers such as poly(vinyl alcohol) (PVA) and poly(ethylene glycol) (PEG) [32]. Although several polymers are evaluated for chitosan polymerization, PEG is of the most interest, owing to its hydrophilicity, biocompatibility and biodegradability [32–34]. In addition, PEG could minimize the interaction between the components due to the character of uncharged polymer [35,36].

PEG-grafted chitosan (PEG-g-chitosan) was prepared by alkylation of chitosan followed by Schiff base formation according to Bhattarai and coworkers [37].

Interpolymeric network gels films based on chitosan were also prepared and crosslinked either chemically with glutaraldehyde or by gamma irradiation [38,39]. In presence of water, radyolisis take place with formation of highly reactive species that can crosslink chitosan with other polymers.

Hydrogels were synthesized in this work based on chitosan biopolymer and polyethylene glycol with different molecular mass, PEG $M_w = 300$ and PEG $M_w = 400$, in order to be used in biotechnology applications.

2. Experimental

2.1. Preparation of the hydrogels

Chitosan solution was prepared in a 500 mL reactor equipped with mechanical stirrer. Chitosan was dissolved in degassed distilled water containing 1 wt.% of acetic acid. Generally, 6.0 g of chitosan were dissolved in 200 mL of distillated degassed 1 wt.% acetic acid solution. After dissolution it was added 1.25 g of PEG 300 or PEG 400 and 4.05 g of AGAR. A continuous stir by 6 h was done. The reactor was placed in a silicone bath preset at 96 °C. After 60 min, the reaction product was allowed to cool at ambient temperature in plastic recipes and neutralized to pH 7 by addition of 1 N NaOH solution.

Those hydrogels were cured by two sources of energy a γ-radiation Co-60 radiation source model 220 IEA activity 6917.4 CL operation at 5.72 kGy/h (25, 20, 15 and 10 kGy) and 60 W UV-radiation lamp (radiation time 45, 90, 180, 300 and 420 min). For UV irradiation, a 60 W UV-radiation lamp was used and samples were poured into a dish and exposed to the full-wavelength UV-radiation for different times (45, 90, 180, 300 and 420 min) at a fixed distance of 17 cm height. Before characterization, the photocopolymerized hydrogels were dried until constant weight.

2.2. Characterization

Characterization of chitosan hydrogels was carried out using infrared spectrometry, thermal analysis, UV–Vis spectroscopy and scanning electronic microscopy (SEM).

The infrared spectra of the samples were used to provide information about the nature of the interaction of chitosan and polyethylene glycol. These spectra were recorded in the range from 4000 to 600 cm^{-1} using ATR sensor by using a NICOLET 100 FTIR spectrophotometer with germanium ATR 1 point. The UV–vis absorption spectra were recorded in the range of 190–800 nm using a Spectrophotometer Shimadzu Model Multispec 1501.

Scanning electronic microscopy were obtained in an Electronic microscopy PHILIPS model XR30, and the films were deposited in sampler and recovered by gold to obtain the desirable contrast.

Thermogravimetric (TG) curves were performed with a SDTA-822 thermobalance (Mettler Toledo), using samples with approximately 7 mg in sapphire crucibles, under dynamic nitrogen atmosphere (50 mL × min^{-1}), at heating rate of 10 °C × min^{-1}.

2.3. Measurement of swelling capacity

The swelling properties (SR) of samples were studied by immersing the samples in a solution and at different periods of time at room temperature. The dried hydrogels were cut into small squares with about 10 mm height. At predetermined time intervals, the disks were taken out from the solution, gently wiped with filter paper to remove the surface solution weighed and returned to the same container until equilibrium. The swelling ratio (SR) of these samples were calculated by using the Eq. (1). [40]:

$$\text{Swelling ratio}(\%) = [(W_s - W_d)/W_d] \times 100, \tag{1}$$

where W_s is the weight of swollen hydrogel and W_d is the weight of dry gel at different swelling time. In order to check the behavior of chitosan hydrogels with the variation of pH it was used four different solutions, acetic acid with pH 2.85, acetic acid/acetate buffer pH 4 a physiologic serum with pH 6.52, water pH 7.0 and ammonium hydroxide solution pH 9.0.

3. Results and discussion

The hydrogels synthesized by two different blends composed by chitosan, polyethylene glycol (PEG 300 and PEG 400) and Agar were exposed to two distinguish sources of energy, γ-radiation (cobalt source with 25, 20, 15 and 10 kGy and 60 W UV-radiation lamp). The hydrogels irradiated by cobalt δ source presented apparent decomposition. They were burned, as shown in Fig. 1. Besides, the hydrogels cured by UV–vis light showed a good performance and appearance. The chitosan could form hydrogels in aqueous solutions due to the physical crosslinking through hydrogen bonding and dipole–dipole interactions between neighboring ester groups and chitosan chains, as well as the hydrophobic side chain aggregation. Under radiation with formation of PEG radicals that combine with chitosan the crosslink could be also possible.

Fig. 2 shows the results of a qualitative method to detect degradation of hydrogel polymers. In the UV absorption spectra of photocopolymerized hydrogels (Fig. 2(c)) is observed homogeneous UV profile. Two peaks are present with maxima at 234 and 374 nm that were attributed to absorption of the matrixes studied. On the other hand the spectra of hydrogels polymerized by γ-radiation showed several peaks, not attributed here, but probably due to any oxidized products originated from decomposition of polymer blends by radiation absorption (Figs. 2(b)–(c)).

The FTIR spectra in absorption mode of dried UV photocopolymerized hydrogels (Fig. 3) showed characteristic bands of chitosan concerned to the ν_{OH} and ν_{NH} centered at 3446 cm^{-1}, Amide I band corresponding to $\nu_{C=O}$ vibration ($\sim$1620–1650 cm^{-1}) of acetyl groups in chitosan, a band attributed to Amide III at $\sim$1380–1310 cm^{-1}, due to combination of NH deformation and the ν_{CN} stretching vibration and the band due to ν_{C-O} at $\sim$1070–1050 cm^{-1}. These bands are shifted when compared with the original bands observed in chitosan spectrum $\nu_{OH}=$ and ν_{NH} (3347) cm^{-1}, $\nu_{C=O}$ (1642 cm^{-1}) ν_{CN}, δ_{NH} 1379–1321 cm^{-1} (shifted), ν_{C-O} at 1071–1043 cm^{-1} corroborating with efficient reticulation of PEGs in chitosan matrix.

For the PEG–chitosan samples, the peaks corresponding to the amino group, hydroxyl and amide group of chitosan are slightly shifted. Also the intensities of the peaks are reduced. These indicated that –NH$_2$ groups of chitosan are partially crosslinked with PEG molecule. The peaks at 1130 cm^{-1} and 2880 cm^{-1} are attributed to the superposition of C–O and C–H stretching vibrations of chitosan and PEG.

The crosslinking are justified by photoradiation mechanism allowed by UV. Radicals formation are promoted in presence of O_2 and oxidized impurities of the chitosan or PEG, under UV-radiation.

Scanning Electronic Microscopy showed in Fig. 4 agrees with homogeneous surface for both photocopolymerized hydrogels and could be observed very little dark regions attributed to porous in the films. It was observed also the samples in decomposition when the incident light reaches the polymer target.

The TGA curves of the PEGs and chitosan–PEG hydrogels are shown in Fig. 5. From those curves, the differences between the PEG and the chitosan–PEG hydrogels can be noted. The first step for losses of mass is from about $T = 31$ to 170 °C for PEG solutions and from about $T = 31$ to 180 °C for PEG–chitosan hydrogels are due mainly to the loss of physically adsorbed water on the PEG solution and hydrogels, respectively. The thermal decomposition of the PEG solutions are observed in the step with mass loss from about $T = 158$ to 690 °C. For chitosan–PEG 300 hydrogel (Fig. 5(a), a unique event of thermal decomposition from about $T = 167$ to 986 °C was observed for all radiation time of this material. When comparing this range of decomposition with the range of PEG and Chitosan (curve not showed) the observed changes are attributed to the reticulation done. Besides, for chitosan–PEG 400 hydrogel, until 90 minutes of irradiation it was found one step of decomposition, from about $T = 157$ to 986 °C and for the material irradiated 3 h were observed two thermal decomposition steps. This agrees with two types of materials volatiles in the composition of hydrogels [41].

The DTGA curves (Fig. 5(b) and (d)) reveal more accurate differences of the thermal behavior of photocopolymerized hydrogels than the TGA curves [42].

The first peak of the DTGA curves of chitosan–PEG 300 photocopolymerized hydrogel in the interval from about $T = 167$ to 986 °C for all irradiation time were related with water loss. The second DTGA peak of chitosan–PEG 300 photocopolymerized hydrogel presents its
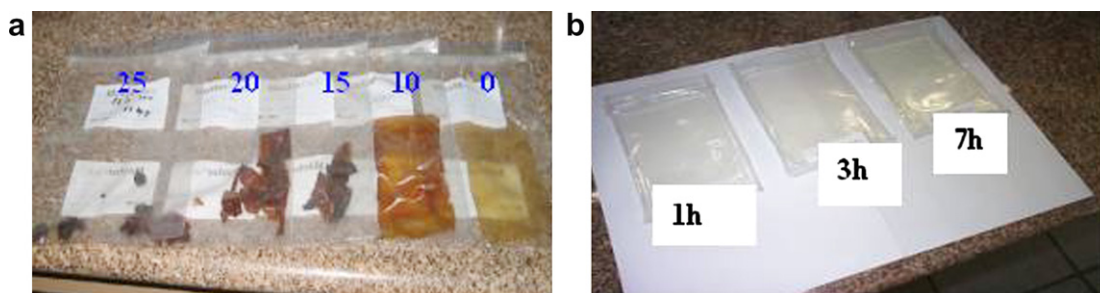

Fig. 1. Chitosan hydrogels membranes irradiated under (a) cobalt source in kGy and (b) UV lamp, in hour.
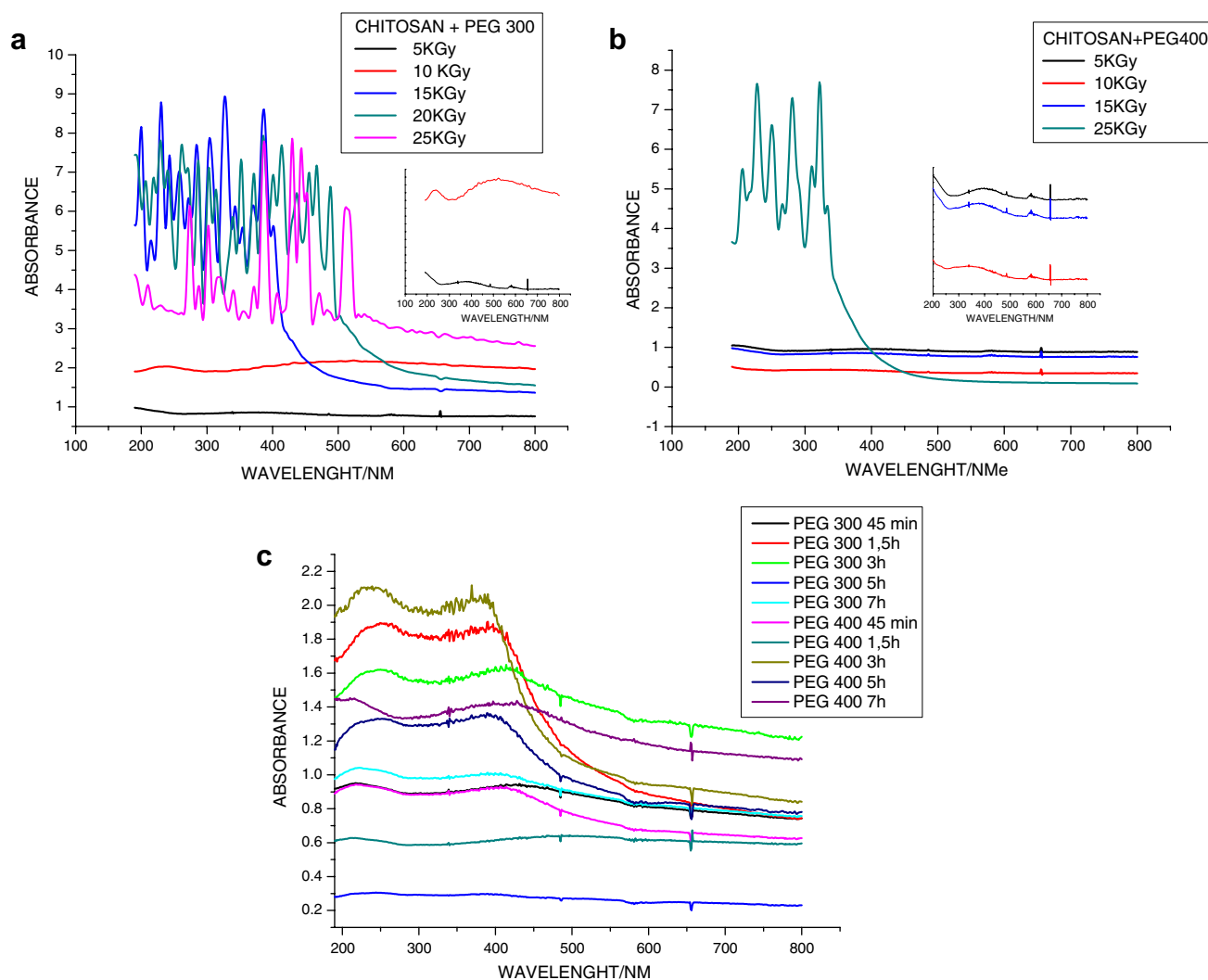
Fig. 2. UV–vis spectra of chitosan gels (a) Chitosan/PEG300, (b) Chitosan/PEG400 under γ-radiation and (c) Chitosan/PEG300/PEG 400 under UV-radiation.
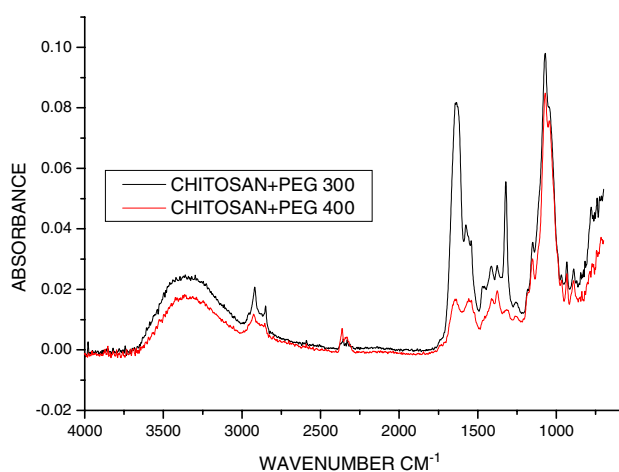

Fig. 3. Infrared spectra of chitosan gels cured by UV light (irradiated 90 min).

maximum value at about $T = 223$, 236, 213, 246 and 219 °C for 45, 90, 180, 300 and 420 min, respectively, while the maximum value of this peak for the chitosan and PEG 300 are observed at about $T = 398$ and 343 °C respectively. The decreasing thermal stability of the chitosan–PEG 300 photocopolymerized hydrogel seems to be related to the formation of different phases on hydrogels during radiation process.

DTGA curves of chitosan–PEG 400 photocopolymerized hydrogel are related with the irradiation time and two types of behavior are found. The first group (45 and 90 min of irradiation) two peaks are recorded and are attributed to adsorbed water in the interval of (31–180) °C and polymer decomposition at 245 and 269 °C for 45 and 90 min, respectively. The second group (3.5, 7 h of irradiation) three peaks are observed and also attributed to adsorbed water in the interval of (31–180) °C and to polymer decomposition the most volatile phase with maximum peaks at 230, 217 and 216 °C (for 3, 5 and 7 h, respectively), and a second peak at 278, 275 and 274 °C (for 3, 5 and 7 h, respectively) attributed to more dense thermostable phase. The decreasing in thermal stability of
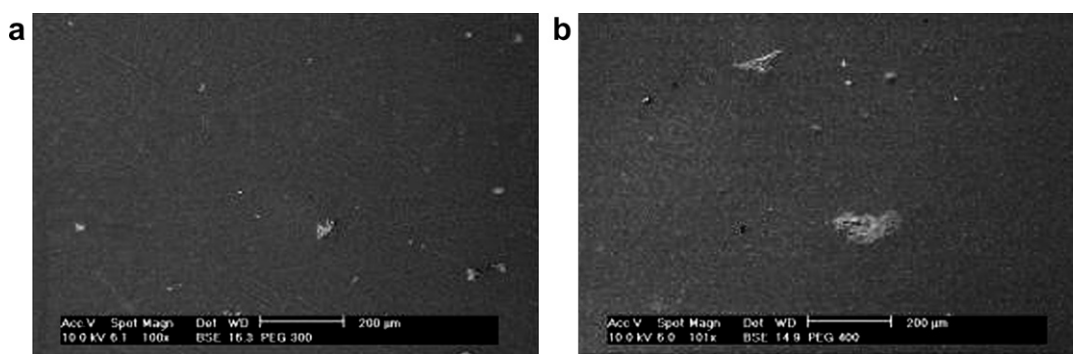
Fig. 4. SEM of Chitosan gels (a) Chitosan/PEG300 and (b) Chitosan/PEG400.
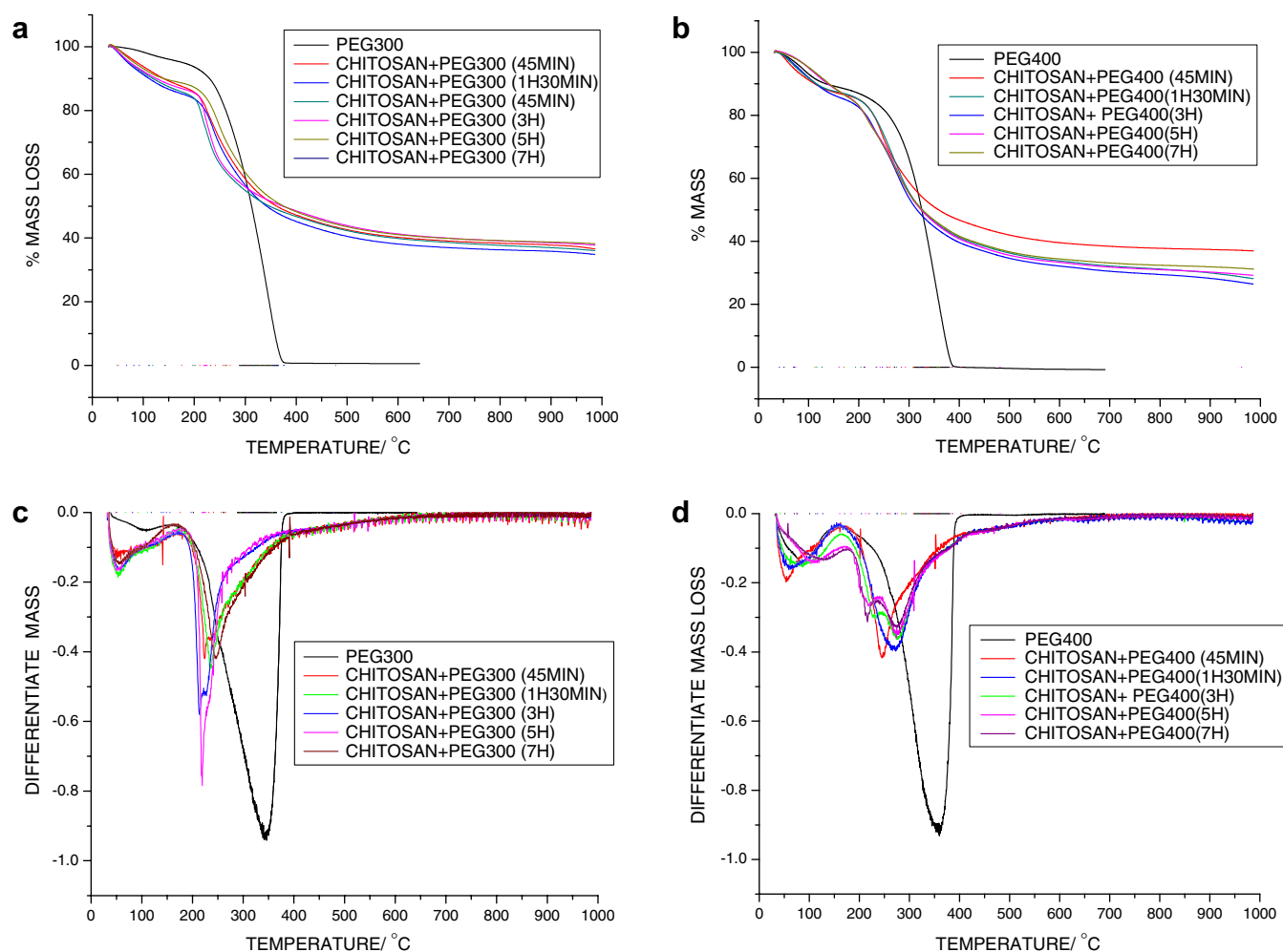

Fig. 5. TGA of Chitosan photocopolymerized hydrogels (a) Chitosan/PEG300, (b) Chitosan/PEG400 and DTGA curves, (c) Chitosan/PEG300 and (d) Chitosan/PEG400.

the chitosan–PEG 400 photocopolymerized hydrogel was also found.

3.1. Swelling of photocopolymerized hydrogels

The equilibrium swelling of hydrogels is a result of the balance of osmotic forces determined by the affinity to the solvent and the network elasticity. Fig. 6 shows the swelling of the photocopolymerized hydrogels of chitosan–PEG 300 and chitosan–PEG 400 irradiated by 45 min, as a function of pH.

According to Fig. 6, the two sharp swelling capacity changes can be attributed to high repulsion of $–NH_3^+$ groups in acidic media and $–COO^-$ groups in basic media. Around pH 5, the carboxylic acid component comes into action as well. Since the pKa of the weak polyacid is about
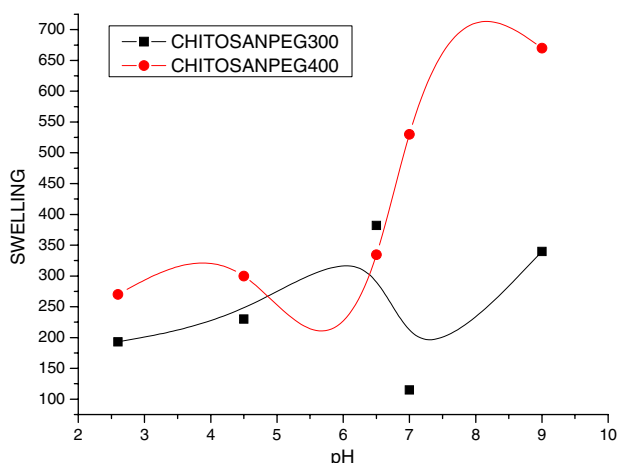
Fig. 6. Swelling behaviors of hydrogels as a function of pH.

Fig. 8. Swelling behaviors of hydrogels as a function of irradiation time.

6.4 [40], its ionization occurring above this value, may favor enhanced absorbance. But under pH 6.4, at a certain pH range 4–6, the majority of the base and acid groups are as non-ionized forms, so hydrogen bonding between amine and carboxylic acid may lead to a kind of crosslinking followed by a decreased swelling. At higher pHs, the carboxylic acid groups become ionized and the electrostatic repulsive force between the charged sites (COO–) causes increasing in swelling.

The hydrogel swelling kinetics, Fig. 7, represent the dynamic swelling behavior of chitosan–PEG 300 and chitosan–PEG 400 photocopolymerized hydrogels in water. Initially, the rate of water uptake sharply increases and then begins to level off. The equilibrium swelling was achieved after 30 min. The swelling corresponds to the crosslinking behavior. In this study, the hydrogel made from chitosan–PEG 300 shows the maximum water-swelling ratio of 115, and the gel from chitosan–PEG 400 shows the maximum water-swelling ratio of 530, respectively for hydrogels with time of radiation equal to 45 min.
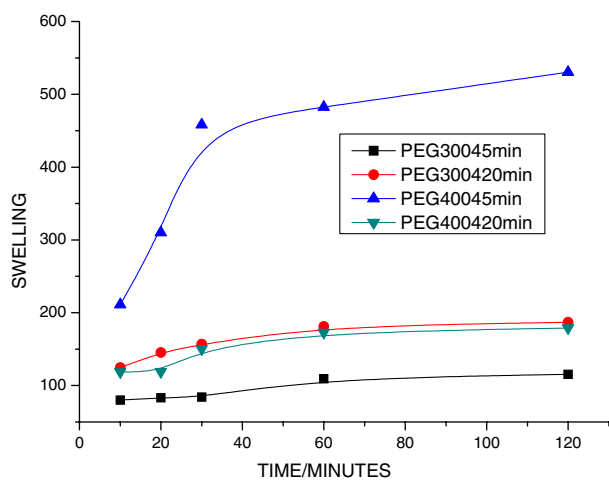
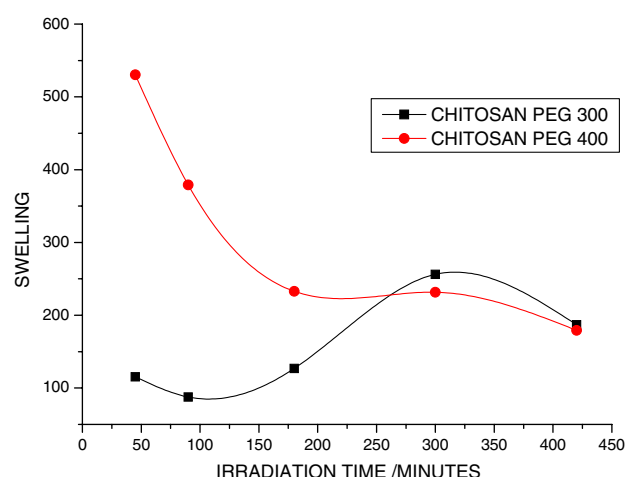## 3.2. Swelling behavior of photocopolymerized chitosan + PEG 300 and Chitosan + PEG 400 blends as a function of irradiation time in the cure process

The equilibrium degrees of swelling of the PEG/chitosan hydrogels as a function of irradiation time are shown in Fig. 8. The swelling curves indicate a typical swelling-dose relationship for PEG 400 and an unusual behavior for PEG 300. The swelling of hydrogels with PEG 400 decreased with the dose, but with PEG 300 the swelling time decreases only when the irradiation time reaches 300 min. As usually swelling is highest just after the gelation dose and decreases with increasing absorbed energy at the early stages of gel formation.

4. Conclusion

A series of novel hydrogels based on PEG and chitosan was synthesized by radiation technology. UV-radiation showed better results than γ-radiation when compared in the crosslink of chitosan hydrogel. Hydrogels characterization agrees with the crosslinking of PEG on chitosan polymer. These hydrogels can provide satisfying properties such as pH-sensitivity, good swelling, potential adsorptive capacity, etc.

The swelling study of photocopolymerized hydrogels showed water absorption up to 530%, into the gels after 2 h. These properties imply that the PEG–chitosan hydrogels, have potential for application in the biomedical field.

Acknowledgments

Fig. 7. Swelling behaviors of hydrogels as a function of time.

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