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In situ chitosan gelation initiated by atmospheric plasma treatment

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ABSTRACT

This work reports on the feasibility of atmospheric dielectric barrier discharge (DBD) plasma as a novel synthetic pathway for the liquid phase gelation of chitosan. The DBD plasma chitosan gelation process did not significantly alter the chemical structure of the biopolymer as confirmed by FTIR study. However, the oxidation processes and local heating effect associated with the solvent evaporation during the plasma treatment could provoke both reaction of chitosan degradation and the cleavage of β-1–4-glycosidic linkages with the concomitant generation of aldehyde groups able to crosslink via Schiff-base with amino groups from other chitosan molecules. Shear viscosity measurements suggested the formation of chitosan fragments of lower molecular weight after the plasma treatment of 1% (w/v) chitosan and fragments of higher molecular weight after the plasma treatment of 2% (w/v) chitosan. The crosslinking density of hydrogels generated during the *in situ* DBD plasma chitosan gelation process increased as a function of the treatment time and concentration of chitosan. As of consequence of the increase of the cross-linking density, the equilibrium swelling ratio and water content decreased significantly.

1. Introduction

Chitosan, a polysaccharide-based cationic copolymer of β-(1→4)-linked 2-acetamido-2-deoxy-D-glucopyranose and 2-amino-2-deoxy-D-glucopyranose, is able to react with many negatively charged surfaces/polymers and also to chelate metal ions. In alkaline solutions above pH 6.5, chitosan forms a hydrated gel-like precipitate due to neutralization of the amine groups and elimination of the repulsive interchain electrostatic forces, allowing intensive hydrogen bonding and hydrophobic interactions. Gelation of cationic chitosan solutions can be achieved even at acidic pH by the addition of polyanions. Gel formation can be further induced by low molecular counterions such as β-glycerophosphate, polyphosphates, sulphates and by crosslinking with dialdehydes such as glyoxal (Khalid, Ho, Agnely, Grossiord, & Couarraze, 1999; Patel & Amiji, 1996), formaldehyde (Singh, Narvi, Dutta, & Pandey, 2006) and in particular glutaraldehyde (Aly, 1998; Denkbas, Seyyal, & Piskin, 2000), despite that the dialdehydes could exhibit problems related to physiological toxicity (Leung, 2001; Murata-Kamiya, Kamiya, Kaji, & Kasai, 1997). Owing to its unique properties such as biodegradability, biocompatibility, high charge density and non-toxicity, the chitosan has great potential for biomedical and pharmaceutical applications either as covalently or ionically cross-linked hydrogel (Bai-Shuan, Chun-Hsu, & Shr-Shin, 2008; Berger et al., 2004; Jahren, Butler, Adams, & Cameron, 2010; Mei-Chin et al., 2009; Muzzarelli, 2009, 2010) as well as blended with other polymers to modulate swelling and mechanical properties (Berger et al., 2004), or combined with thermo-responsive polymers to exhibit stimuli-responsive properties (Lee, Wen, Lin, & Chiu, 2004; Prabaharan & Mano, 2006; Sai-bo et al., 2010).

Recently, *in situ* forming hydrogel systems based on liquid phase plasma polymerization (Baroch, Anita, Saito, & Takai, 2008; Joshi, 2010) have been reported. Liquid phase plasma polymerization can be performed without the use of chemical initiators or/and even without cross-linking agents, offering multiple advantages over the conventional hydrothermal and chemical polymerization methods. Precursors or monomers with a high vapour pressure can also be used since no volatile compounds are needed in contrast with the plasma polymerization in gas phase. Different experimental configurations can be employed for the generation of non-thermal plasmas in liquid and/or in contact with liquids, e.g. direct liquid phase discharges, discharges in the gas phase with liquid electrode(s), and discharges in bubbles in liquids. Discharges in liquids and in contact with liquids generate UV radiation, shock waves and reactive species than could promote oxidative and degradation

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processes of pollutants, biological material and are also very effective in modifying material surfaces (Baroch et al., 2008; Bruggeman & Leys, 2009; Joshi, 2010). Glow discharge electrolysis and liquid phase capillary discharge have been used to obtain functionalized polymer surfaces with tailored chemical functionality such as carboxylic groups (Gao et al., 2008; Joshi, Dieter-Schulze, Meyer-Plath, Wagner, & Friedrich, 2009; Joshi, Friedrich, & Wagner, 2009). In addition, a specific solution plasma system was introduced as a method for the preparation of low molecular weight chitosan and chito-oligosaccharides (Montembault, Viton, & Domard, 2005; Prasertsung, Damrongsakkul, Terashima, Saito, & Takai, 2012). In this work, a novel *in situ* liquid phase dielectric barrier discharge (DBD) plasma method for the synthesis of chitosan hydrogels, emphasizing the influence of the plasma treatment time and chitosan concentration on physico-chemical characteristics of the generated hydrogels is proposed. The study performed herein would be of relevance for plasma assisted gelation of chitosan also on polymer or textile substrates.

2. Experimental

2.1. In situ plasma gelation of chitosan

A dielectric barrier discharge (DBD) reactor operating at atmospheric pressure was used in this work (Fig. 1). Gas mass flow metre and controllers (Bronkhorst, Ruurlo, Netherlands) were used in order to introduce helium gas (5 L_n min^{-1}) in the reactor chamber. A 100 kHz signal was generated with a GF-855 function generator (Promax, L'Hospitalet de Llobregat, Spain) connected to a linear amplifier AG-1012 (T&C Power Conversion Inc., Rochester, NY, USA).

The incident power in the plasma reactor was kept constant at 40 W. A matching network and two transformers (HR-Diemen S.A., Sant Hipòlit de Voltregà, Spain) were connected to the amplifier output in order to increase the voltage up to ≈20 kV. The distance between the two electrodes was kept constant approximately at 5 mm. A small amount (approximately 4 ml) of chitosan solution was poured into a polystyrene Petri dish and has been placed between the electrodes. The 1% and 2% (w/v) solutions of medium molecular weight chitosan (190–310 kDa determined by viscosity, Sigma Aldrich) and degree of deacetylation of 85% were freshly prepared under an overnight stirring in a 1% (v/v) acetic acid. Thereafter, the chitosan solutions were filtered off through a 0.45 μm Millipore filter to eliminate impurities. *In situ* DBD plasma chitosan gelation was carried out during 15, 25 and 35 min.

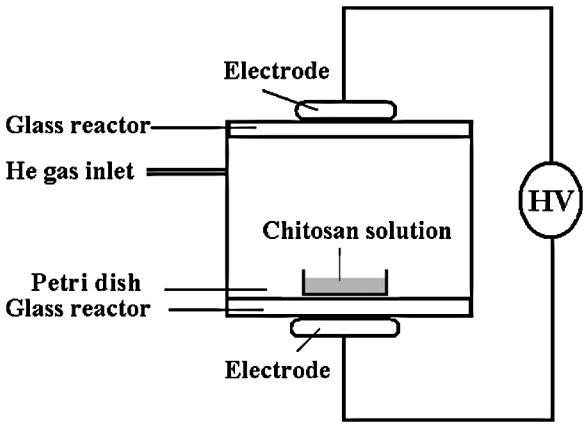


Fig. 1. Experimental set-up for *in situ* liquid phase preparation of chitosan hydrogels initiated by DBD plasma.

2.2. Characterization of chitosan hydrogels

2.2.1. Qualitative evaluation of gelation/crosslinking efficiency

The qualitative gelation/crosslinking efficiency of chitosan hydrogels has been evaluated by their re-solubilisation rate in aqueous solution of acetic acid (1%, v/v) at room temperature (22 ± 2 °C). The weight change (%) of hydrogels was monitored as a function of the immersion time (15 min, 1, 4 and 24 h) in fresh acetic acid (1%, v/v) solution.

2.2.2. Quantitative evaluation of gelation/crosslinking efficiency

The equilibrium swelling ratio, ESR (%) measured gravimetrically was calculated according to the equation:

$$\text{ESR}\ (\%) = \left[\frac{W_s - W_d}{W_d}\right] \times 100 \quad (1)$$

where W_d is the weight of dried chitosan hydrogels, and W_s is the weight of chitosan hydrogels immersed in acetic acid (1%, v/v) water solution for 24 h.

The equilibrium water content, EWC (%) was calculated from the equation:

$$\text{EWC}\ (\%) = \left[\frac{W_e - W_d}{W_e}\right] \times 100 \quad (2)$$

where W_d is the weight of dried chitosan hydrogels, and W_e is the weight of chitosan hydrogels in the equilibrium swollen state i.e. after 24 h immersion in aqueous acetic acid solution at room temperature. The equilibrium water content actually stands for the equilibrium content of aqueous acetic acid solution in chitosan hydrogel.

2.2.3. FTIR analysis

The infrared (IR) spectra were recorded by a Nicolet AVATAR 360 FTIR spectrometer operating in the transmission mode. KBr pellets were prepared with a powdered freeze dried chitosan hydrogels, whereas the FTIR of plasma treated solutions were prepared using a horizontal ATR unit for liquids. In the same way, FTIR spectra of chitosan films obtained by simple casting method (60 °C overnight) were also prepared for comparative purposes. Spectra were normalized to the band at 1070 cm^{-1} corresponding to C—O stretching vibration in chitosan. Background was extracted by means of linear backgrounds performed between 1800 and 1463 cm^{-1}, 1463 and 1354 cm^{-1}, 1354 and 1278 cm^{-1}, 1278 and 1216 cm^{-1}, and 1216 and 850 cm^{-1}. A total of 32 scans were recorded for each measurement at a resolution of 4 cm^{-1}.

2.2.4. SEM analysis

The morphology of chitosan hydrogels (air-dried at room temperature or freeze dried) was studied by scanning electron microscopy (model Hitachi S-3500N). The swollen gel was quickly frozen with liquid nitrogen and then freeze-dried for 24 h prior to SEM observation. Samples were coated with Au/Pd (thickness coating ~20 nm) in a sputtering device Polaron SC500 prior to SEM observation.

2.3. Characterization of plasma treated chitosan solutions

2.3.1. Shear viscosity measurement

Shear viscosity of untreated and plasma treated chitosan solutions were measured by AR-G2 Magnetic Bearing Rheometer (TA Instruments, USA). Cone geometry with a cone angle of 4° and radius of 40 mm was used for the measurements at a gap size of 105 μm. The shear rate ranged from 0.01 to 100 s^{-1}, with 5 data points acquisition per decade. All experiments were conducted at temperature of 25 °C. Steady-state shear viscosity is reported as a function of shear rate applied.

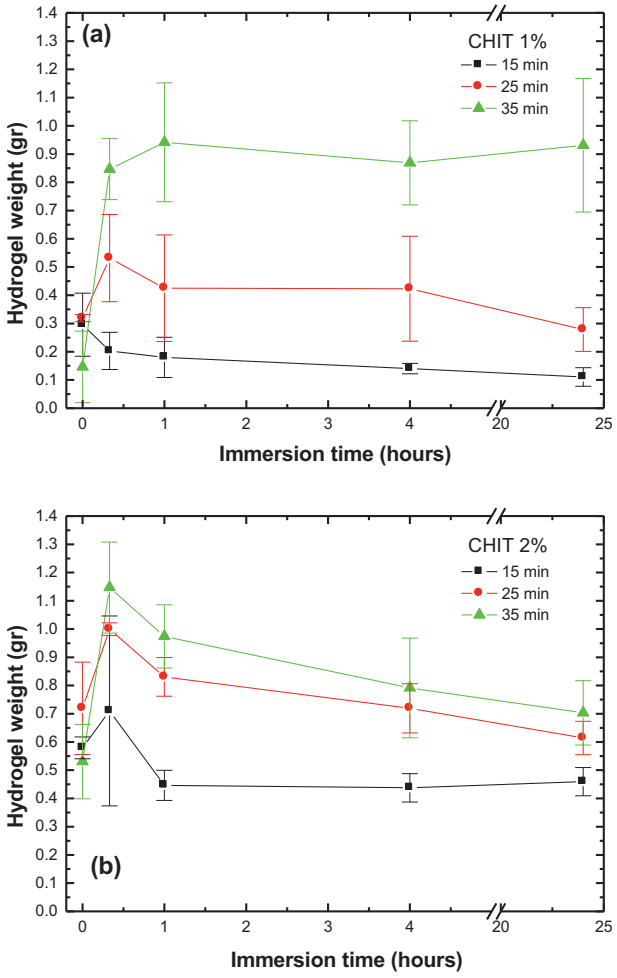


Fig. 2. The hydrogel weight as a function of immersion time, plasma treatment time and chitosan concentration: (a) 1% CHIT and (b) 2% CHIT.

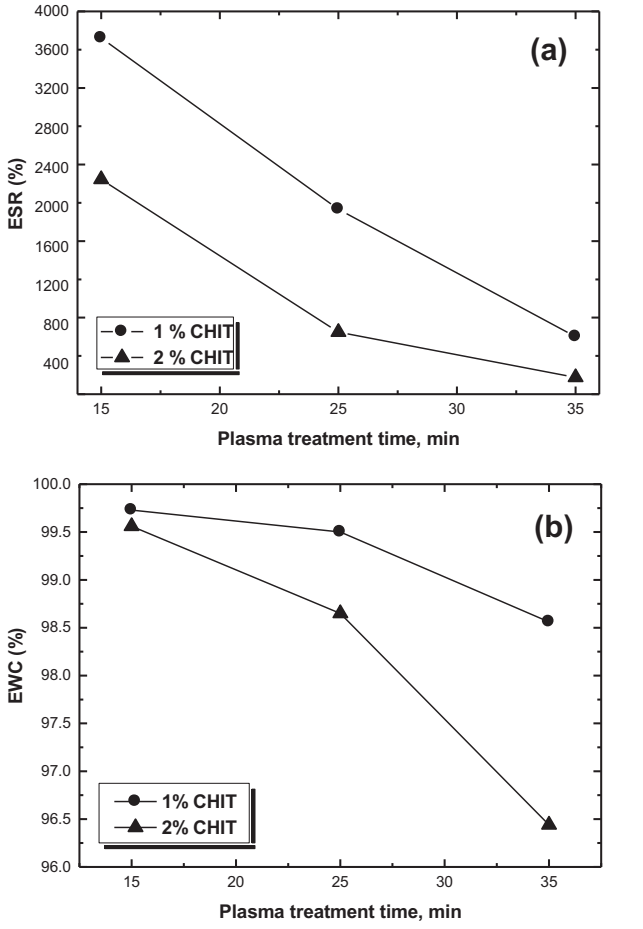


Fig. 3. (a) Equilibrium swelling ratio (ESR) and (b) equilibrium water content (EWC) as a function of CHIT concentration and plasma treatment time.

3. Results and discussion

3.1. Gelation/crosslinking efficiency of chitosan hydrogels

The exposure of chitosan acetic acid aqueous solutions (CHIT) to atmospheric dielectric barrier discharge (DBD) plasma results in gelation of chitosan. As a function of plasma treatment time, two different DBD plasma regimes (filamentary regime and diffuse dielectric barrier discharge regime) can be established closely related to the presence or absence of water in liquid phase inside the plasma reactor. At short plasma treatment times (up to 15 min) a thin hydrogel film is formed on chitosan solution surface. The amount of hydrogel formed increases as a function of plasma treatment time simultaneously with a decrease in chitosan solution volume due to solvent evaporation. At long plasma treatment times (over 30 min) the solvent has been almost completely evaporated and the chitosan hydrogel starts to dry progressively until a dry chitosan film is obtained (≈35 min). Therefore, in the current work, three different plasma treatment times (15, 25 and 35 min) have been intentionally employed to characterize chitosan gelation/crosslinking process.

To qualitatively evaluate the gelation/crosslinking efficiency of chitosan hydrogels formed during DBD plasma treatment, their re-solubilisation rate in acetic acid aqueous solutions was gravimetrically measured. As observed in Fig. 2a and b, the quantity of chitosan hydrogel formed (represented by zero immersion time) depends on both, plasma treatment time and chitosan concentration. After initial swelling and an increase in hydrogel weight, the amount of hydrogel gradually decreased over 24 h of immersion, due to partial dissolution of non-gelated chitosan. Nevertheless, the final amount of the hydrogel measured indicates that certain quantity of chitosan is irreversibly gelated/cross-linked. The longer the plasma treatment time, the lower the re-solubilisation rate of chitosan hydrogel is. In other words, the swelling effect prevails over dissolution of chitosan if plasma treatment time is over 25 min (independently on the chitosan concentration). At shorter plasma treatment times (<15 min) the final weight of chitosan hydrogel is lower comparing to the initial one, suggesting great extent of dissolution of chitosan hydrogel and therefore a poor gelating/crosslinking effect.

The relatively large experimental errors in measuring chitosan hydrogel weight could be explained by the assumption that a complex mixture of gelated/cross-linked material, ungelated/uncross-linked chitosan molecular chains and lower molecular weight chitosan fragments could be formed during *in situ* plasma treatment of chitosan solutions. In addition, this could imply that the gelation process is inherently heterogeneous due to the complex phenomena occurring at the interface of chitosan solution and plasma active species.

3.2. Swelling properties of hydrogels

The equilibrium swelling ratio (ESR) and equilibrium water content (EWC) of chitosan hydrogels are presented in Fig. 3. The ESR and EWC values were highly influenced by the plasma treatment time and concentration of chitosan. The chitosan

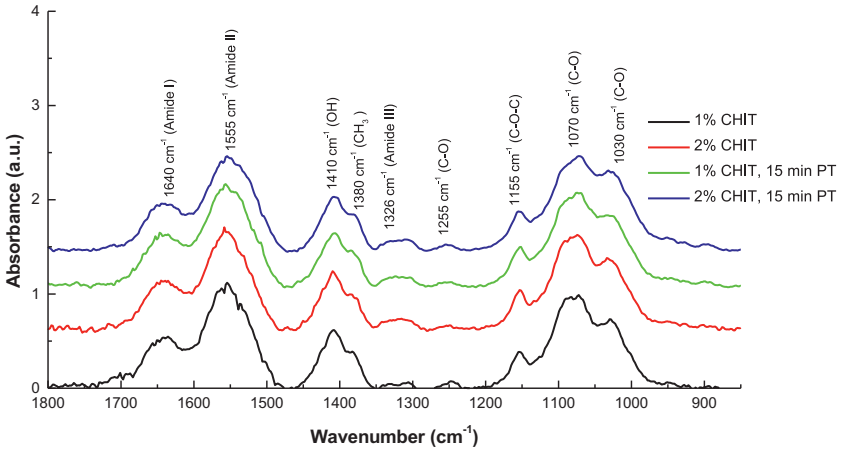


Fig. 4. FTIR spectra of untreated and plasma treated chitosan solutions (PT 15 min).

hydrogels obtained at lower chitosan concentration and plasma treatment time were able to absorb significant quantity of aqueous acetic acid solutions. As plasma treatment time increases, EWC of chitosan hydrogels and swelling capacity decrease, presumably due to an increase in gelation/crosslinking density as a result of chemical bonds formation between chitosan polymeric chains. The decreased swelling capacity of chitosan hydrogels could be also related to the slower relaxation time of the polymeric chains with an increase in crosslinking density (Mi, Kuan, Shyu, Lee, & Chang, 2000).

Restricted freedom of movement of chitosan macromolecules in more concentrated solutions can additionally intensify the crosslinking density obtained by means of plasma treatment. The Brownian motion of chitosan polymer chains at the solution surface decreases with increasing of chitosan concentration as plasma treatment apparently affects the upper part of the solution. Both, the higher residence time of the molecules in the upper solution layer and the decrease of distance between polymer chains in more concentrated chitosan solutions would favour the gelation/crosslinking process. Therefore, it could be possible to control the gelation/crosslinking density and swelling properties of chitosan hydrogels by proper modulation of plasma treatment time and chitosan concentration.

3.3. Chemical characterization of chitosan hydrogels and plasma treated solutions

FTIR spectra in the fingerprint region (1800–850 cm^{-1}) of untreated and plasma treated chitosan solutions (Fig. 4) were recorded to study possible chemical or physical processes involved in chitosan gelation during plasma treatment. The spectrum of untreated chitosan shows peaks around 897 and 1149 cm^{-1} corresponding to the glycosidic bridge present in saccharide structure. There are several peaks clustering in the amide peak range from 1300 to 1700 cm^{-1}, denoted as amide I (1640 cm^{-1}), amide II (1555 cm^{-1}) and amide III (1326 cm^{-1}). Signals corresponding to OH in the ring (1410 cm^{-1}) CH_3 groups in amide group (1380 cm^{-1}) and C—O group (1255 cm^{-1}) can be observed in the central part of the spectrum. The broad peaks at 1030 and 1070 cm^{-1} indicate the C—O stretching vibration in chitosan (Pawlak & Mucha, 2003; Shigemasa, Matsuura, Sashiwa, & Saimato, 1996; Sinitsya, Copıkova, Prutyanov, Skoblya, & Machovic, 2000; Zawadzki & Kaczmarek, 2010). No significant difference can be observed between untreated chitosan and chitosan solutions treated with plasma for a plasma treatment time of 15 min, suggesting the partial gelation of chitosan occurs predominantly in the upper part of the solution which is in intimate contact with the plasma active species, also confirming the results of ESR and EWC analysis.

However, the FTIR spectra corresponding to the chitosan hydrogels obtained after 35 min of plasma treatment (Fig. 5) shows noticeable differences respect to untreated chitosan and plasma treated solutions which can be attributed to the complex gelation mechanism induced by the plasma treatment. The spectra corresponding to chitosan hydrogels obtained after 35 min plasma treatment evidence a decrease in intensity of bands at 1640, 1555 and 1410 cm^{-1} that can be associated to both physically absorbed water and hydrogen bonded molecules (Desbrieres, 2002; Rivero, García, & Pinotti, 2012). A new band around 1710 cm^{-1} attributed

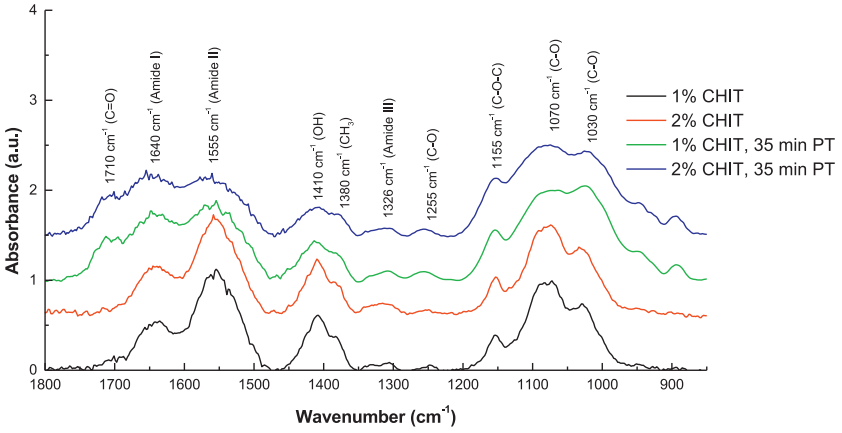


Fig. 5. FTIR spectra of chitosan films and hydrogels obtained after 35 min of plasma treatment (PT 35 min).

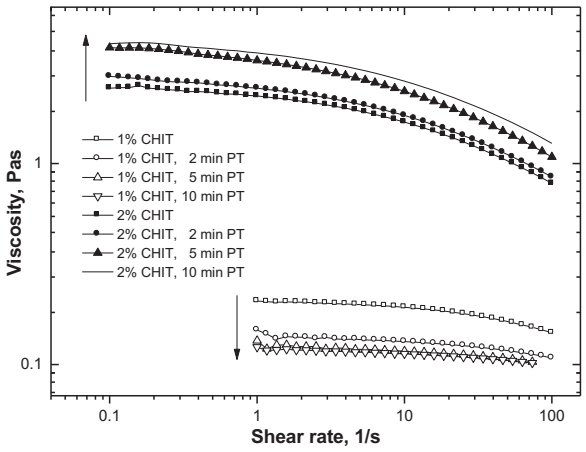


Fig. 6. Shear rate dependence on viscosity for untreated and plasma treated chitosan solutions (1% CHIT and 2% CHIT).

to carbonyl moieties (aldehyde groups), absent in the FTIR spectra of plasma treated chitosan solutions and chitosan films, clearly appeared in spectra of chitosan hydrogel obtained after 35 min of plasma treatment.

Additionally, an intensity increment of the C—O band at 1030 cm^{-1} could suggest a possible oxidation of D-glucopyranose rings and breakage of β-1–4-glycosidic linkages (Vaideki, Jayakumar, Thilagavathi, & Rajendran, 2007). As a result, the molecular weight of chitosan rather than the chemical composition of the polymer could be affected during the plasma chitosan gelation process. The hydroxyl radicals formed during the chitosan solution plasma treatment are able to break the β-1–4-glycosidic linkages leading to the formation of chitosan fragments of lower molecular weights (Chang, Tai, & Cheng, 2001; Prasertsung et al., 2012; Vaideki et al., 2007) capable also of Schiff-base inter and intra crosslinking reactions between the aldehyde and amino groups from other chitosan molecules due to their high reactivity (Prasertsung et al., 2012; Zotkin, Vikhoreva, Smotrina, & Derbenev, 2004).

Given the existence of plasma filamentary regime when aqueous solutions are present inside a plasma reactor, significant solvent evaporation is observed due to local heating of the chitosan solutions. Therefore, at prolonged plasma treatment times (over 15 min), similar effects to that obtained by the thermal modification of chitosan (N-acetylation, degradation and crosslinking reactions) could be expected. Nevertheless, N-acetylation reaction was not confirmed in our study suggesting that the oxidation processes

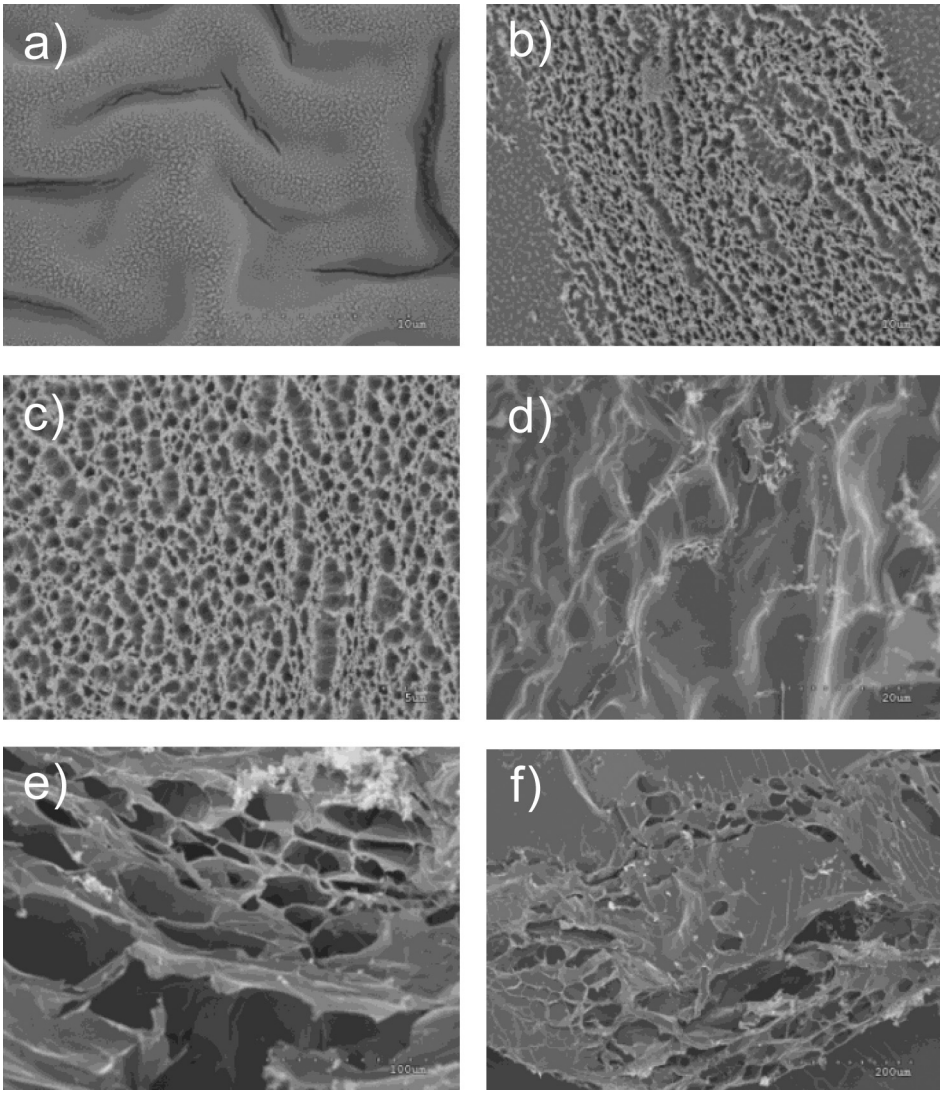
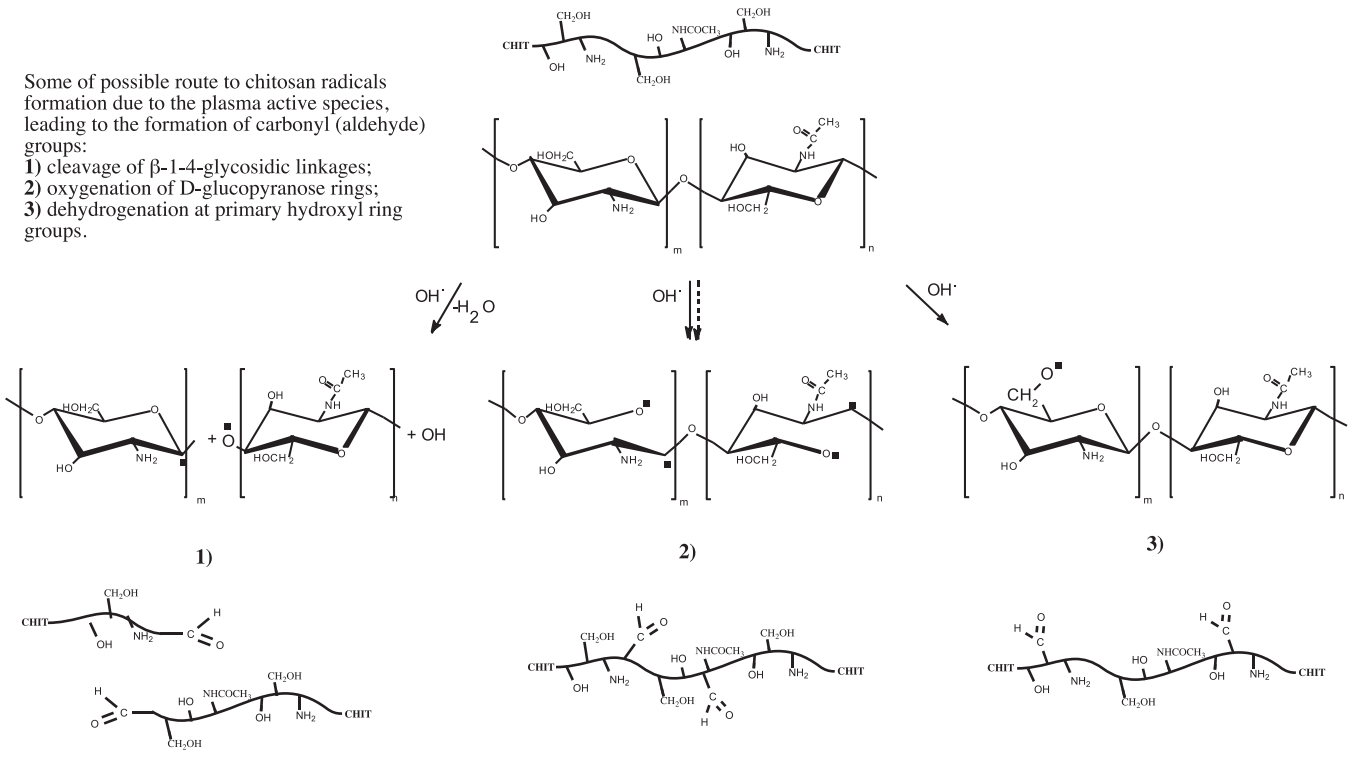


Fig. 7. SEM images of CHIT prepared by different drying methods: (a–c) air-dried CHIT hydrogels obtained after 35 min of plasma treatment; (d–f) freeze-dried swollen CHIT hydrogel obtained after 35 min of plasma treatment.

Some of possible route to chitosan radicals formation due to the plasma active species, leading to the formation of carbonyl (aldehyde) groups:
1) cleavage of β-1-4-glycosidic linkages;
2) oxygenation of D-glucopyranose rings;
3) dehydrogenation at primary hydroxyl ring groups.



DBD plasma gelation/crosslinking of chitosan

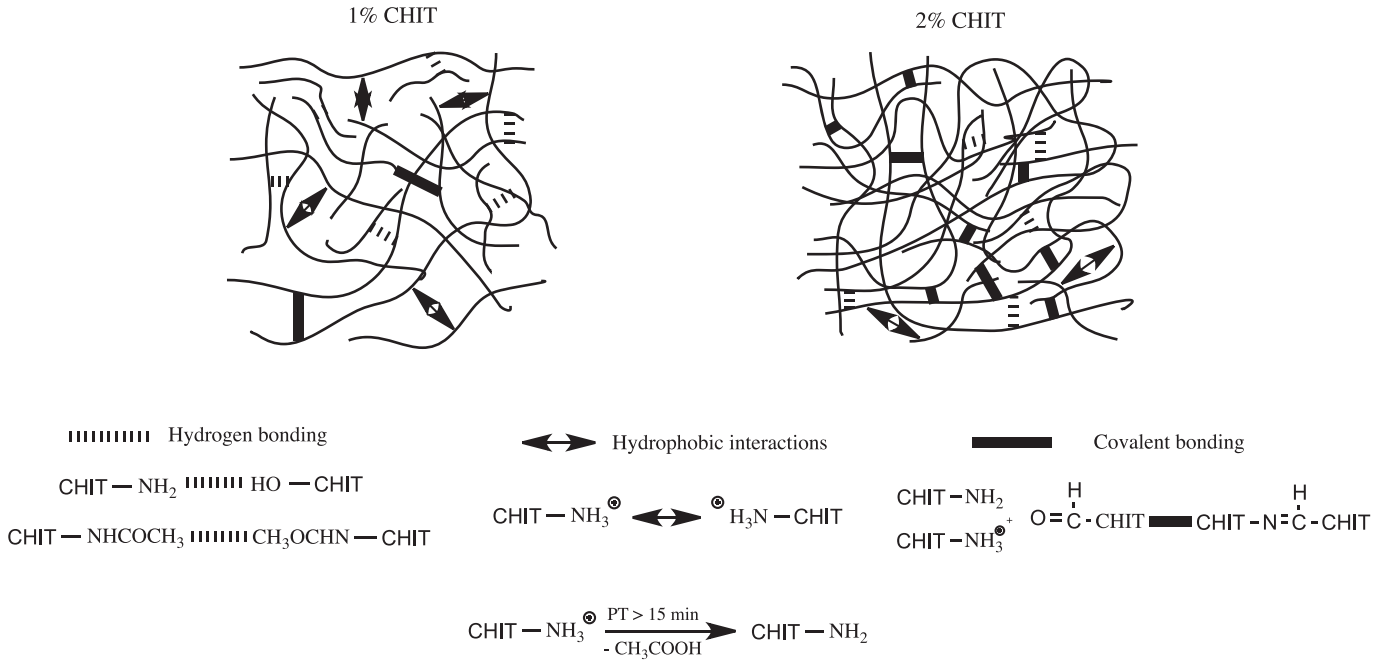


Fig. 8. Simplified scheme of CHT gelation/cross-linking process.

seem to be dominant during the chitosan gelation, particularly at prolonged plasma treatment. General appearance of FTIR spectra of hydrogels, treated chitosan solutions and untreated chitosan also imply that the plasma gelation process did not significantly affect the chemical structure of polymer.

The rheological properties (shear viscosity measurements) of chitosan plasma treated solutions were also determined for chitosan solutions up to 10 min of plasma treatment time (Fig. 6). It was observed that shear viscosity of 1% chitosan solution decreased as a function of plasma treatment time, while in the case of 2% chitosan solution, an increase in viscosity was detected.

At the lower chitosan concentration used (1% CHIT), the viscosity decrease can be attributed to the partial degradation of the chitosan biopolymer via oxidation processes and appearance of lower molecular weight chitosan fragments as previously suggested by FTIR analysis. The Newtonian behaviour of chitosan plasma treated solutions is extended to higher values of shear rates, supporting that low molecular weight chitosan fragments are formed. At the

higher chitosan concentration used (2% CHIT), the reduction of the Newtonian region at low shear rates and an increase in viscosity as a function of plasma treatment time, suggest possible formation of conjugates of higher molecular weights in the solution by Schiff-base inter- and intra-cross-linking reactions.

3.4. Morphology of chitosan hydrogels

The morphology of room temperature or air-dried and freeze-dried hydrogel obtained after 35 min of plasma treatment was analyzed by SEM (Fig. 7). Fig. 7a–c demonstrated that the room temperature dried hydrogel has a hierarchical rough surface pattern consisting in micro- and nano-scale spot-like roughness. The micro-scale surface roughness could be attributed to dissipative solitons spots with characteristic architecture that depends on the frequency of the plasma source used (Molina, Hidalgo, Jovancic, & Bertran, 2013; Stollenwerka, Gurevich, Laven, & Purwins, 2007). However, spot-like nano-scale roughness could be attributed to plasma filament impact on polymer surface resulting in locally higher temperature that could promote polymer melting or surface etching of polymeric material during plasma treatment.

The freeze-dried swollen hydrogels have presented more porous structure unlike the room temperature dried chitosan hydrogels (see Fig. 7d–f). From the Fig. 7, it seems that hydrogel morphology depends on drying method used for preparation of the samples. The differences between freeze-dried and room temperature-dried hydrogels were rather due to the quick freezing of the swollen gel and subsequent evaporation of the solvent than the plasma process itself.

Taking all results into the consideration, a simplified scheme of chitosan gelation/cross-linking process is proposed (Fig. 8). As known, in aqueous acid solution (pH value 2.8), chitosan behaves as a cationic polyelectrolyte due to protonation of the amino groups.

The presence of protonated amino groups is the main reason for existence of hydrophobic interactions (i.e. NH_3^+ and $-NH_3^+$ electrostatic repulsions) between the structural units of chitosan. These hydrophobic interactions led to the increase in the osmotic pressure inside the hydrogel network. Finally, the osmotic pressure difference between the internal and external aqueous acidic acid solution is balanced by the excellent hydrogel swelling properties (see Fig. 3a, ESR and EWC values). Meanwhile, the presence of acetylated chitosan units (its number depends on degree of deacetylation) results in hydrogen intra- and inter-bonding of chitosan chains. During the plasma treatment, solvent evaporation and local heating of the chitosan solutions is observed at time over 15 min, which could lead to the partial de-protonation of amide groups (Fig. 8) and establishment of additional hydrogen bonding with hydroxyl groups of other chitosan molecules.

During the plasma treatment (bombardment of charged ions on the surface of chitosan aqueous solution), the reactive H, OH radicals and the solvated electrons are formed. These reactive species react with the chitosan leading to the generation of carbonyl (aldehyde) groups predominantly by oxygenation of D-glucopyranose rings, cleavage of β-1–4-glycosidic linkages and dehydrogenation at primary hydroxyl groups (Prasertsung et al., 2012; Vaideki et al., 2007). The appearance of lower molecular weight chitosan fragments after the plasma treatment of 1% CHIT is confirmed by FTIR analysis and viscosity decrease. As plasma time increases, an increase in gelation/cross-linking density is observed due to formation of covalent bonds by Shift-base reactions between chitosan polymeric chains (Fig. 8) particularly at the higher chitosan concentration used (2% CHIT), as confirmed by reduction of the Newtonian region at low share rates and an increase in viscosity (the appearance of conjugates of higher molecular weights in the solution). We believe that the higher residence time of the molecules in the upper solution layer and the decrease of distance between polymer chains in more concentrated chitosan solutions, significantly favour the gelation/cross-linking process, impacting the swelling capacity of hydrogel obtained. In conclusion, the chitosan gelation/cross-linking process initiated by atmospheric plasma could be of great relevance in preparing of hydrogels for prospective therapeutic applications, polymer and textile coatings.

4. Conclusions

This study reports on the straightforward gelation of chitosan solutions upon application of atmospheric dielectric barrier discharge (DBD) plasma. The general similarities in the FTIR spectra of hydrogels, plasma treated chitosan solutions and untreated chitosan imply that the plasma gelation process did not significantly alter the chemical structure of the biopolymer. However, the plasma oxidation processes together with the local heating effects and solvent evaporation during the plasma filamentary discharges could provoked the gelation/cross-linking of chitosan by Schiff-base reactions between chitosan fragments derived from possible cleavage of β-1–4-glycosidic bonds and oxidation of D-glucopyranose rings. Shear viscosity measurements confirmed the formation of chitosan fragments of either lower or higher molecular weight depending on the chitosan initial concentration during the plasma reaction. The cross-linking density of chitosan hydrogels generated *in situ* by DBD plasma treatment increased as a function of the treatment time and concentration of chitosan. Consequently, the equilibrium swelling ratio and equilibrium water content decreased significantly. Control of the cross-linking density and swelling properties of hydrogels could be achieved by the proper modulation of plasma reaction time and chitosan concentration. *In situ* gelation of aqueous solution of chitosan initiated by atmospheric DBD plasma treatment could be a viable novel pathway for hydrogel synthesis and/or polymeric and textile coatings.

Acknowledgements

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