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Hydrogels from Chitosan and a Novel Copolymer Poly(*N*-Vinyl-2-Pyrrolidone-*Co*-Acrolein)

Janaina Aline Galvão Barros*, Antonio Jedson Caldeira Brant, Luiz Henrique Catalani

Institute of Chemistry, University of São Paulo, São Paulo, Brazil
Email: catalani@usp.br, *janabarros@usp.br

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ABSTRACT

PVP, a synthetic polymer and chitosan, a natural polymer are biocompatible and have presented a great variety of interesting properties for cosmetic, biomedical, pharmaceutical and biotechnological applications, Many alternatives to improve the polymer propeties has been made as bleeding polymers, for example. In this work, two different techniques of hydrogel attainment were used: one from mixtures of acid aqueous co-solutions of chitosan and PVP, whose resultant films and solutions were irradiated, afterwards, by ultraviolet radiation (λ = 254 nm), another one from the reaction of poly (N-vinyl-2-pyrrolidone-co-acrolein)-a novel copolymer synthesized in our biomaterials laboratory-and chitosan. In the first one alternatives it was possible produce hydrogels directly from mixtures of aqueous acidic co-solutions of both polymers. In the second one, the attainment of hydrogels from Schiff base has proved to be an effective methodology for the production of hydrogels, showing good values of gel content and swelling.

Keywords: *Chitosan*, *Hydrogel*, *Copolymer*, *Crosslinking*, *Schiff Base*, *Poly*(*Vinyl-Pyrrolidone-Co-Acrolein*)

1. Introduction

The search for novel biomaterials, mainly polymer hydrogels, is increasing throughout the world. In 1960, Wichterle and Lim developed the first synthetic polymer hydrogel based on 2-hydroxyethyl methacrylate (2-HEMA) as the hydrophilic monomer [1]. Throughout the last five decades numerous publications on this research topic have been released. Hydrogels play an important role in tissue engineering [2-5], dressings for burn wounds and other kinds of injuries [6,7], "intelligent" hidrogels for drug controlled release [8,9], and other biomedical applications [10].

Hydrogels are three-dimensional hydrophilic polymer networks capable of swelling in water or biological fluids and retaining a great amount of fluid in the swollen state without dissolution of their structure [11,12]. They can be obtained through chemical and physical processes [13]. Dressings based on polymeric hydrogels show many advantages, such as exudate absorption, immediate pain relief, barrier to microorganisms, permeability to oxygen, adjustable transparency and mechanical properties [14].

Poly(*N*-vinyl-2-pyrrolidone) (PVP) is a synthetic linear non-toxic, biocompatible polymer, frequently used in food and cosmetics industries as well as in pharmaceutical formulations [15,16]. It is very soluble in water and many organic solvents. Its use as a biomaterial in artificial blood plasma was prevalent in World War II [17-19]. Nevertheless, over the last five decades, PVP has found other applications of greater scientific-biotechnological interest, such as hydrogels for drug controlled release [20,21], tissue regeneration and implants [22], wound and burn dressings [23], and others applications. Hydrogel dressings have attracted much attention among researchers for their use in the medicinal field, chiefly in the healing of burn wounds. It must be emphasized here that there are already numerous natural and synthetic polymers being studied and/or applied as medicinal hydrogels, and PVP is among them [24,25]. But, beyond PVP as a unique polymer, other polymer systems involving VP-monomers are also used in this application area: chemically modified PVP [26-28], copolymers containing units of *N*-vinyl-2-pyrrolidone in their chains for example, poly(methacrylamide-*co*-*N*-vinyl-2-pyrro-lidone-*co*-itaconic acid) [29]; poly(*N*-vinyl-2-pyrrolidone-*co*-styrene) [30]; poly(*N*-vinyl-2-pyrrolidone-*co*-acrylic acid), [31] and blends of PVP with other biocompatible polymers (for example, PVP-CMC [32]; PVP-PVA [33]; PVP-k-carrageenan [34]; PVP-chitosan [35].

Chitosan is another polymer that has found use in biomedical applications over the last three decades [36]. Chemically, it is constituted of units of 2-amine-2-deoxy-D-glycopyranose and 2-acetamide-2-deoxy-D-glycopyranose linked by β- (1→ 4) glycoside linkages [37] along a linear chain. Commercially, it is obtained from alkaline deacetylation of chitin through a hydrolysis reaction using an aqueous solution with ca. 50% KOH at temperatures near to 100˚C. Chitin is a linear polysaccharide present mainly in exoskeletons of crustaceans (shrimps, crabs, lobsters, etc.) [38]. After cellulose, it is the most abundant natural biopolymer on the earth [39]. Thus, chitosan is a renewable and sustainable material. Also, due to its biocompatibility, non-toxic properties and it having inhibitory effects on the growth of fungi and bacteria, there is a growing interest in its potential application in the biomaterials field.

Chitosan is practically insoluble in water and organic solvents. Although it is a hydrophilic polymer, its dissolution in water only takes place in dilute acidic solutions, in which organic acids (for example, formic acid, acetic acid) or mineral acids (for example, HCl, HNO_3) are utilized. These protonate its available amine groups, leading to the formation of a hydrosoluble polycation at pHs < 6. Solubilization of chitosan constitutes, therefore, an important step for its use in diverse types of biomaterials.

Chitin derivatives containing more than 50% of free amine in their structure are usually denominated chitosan [40]. The majority of commercial chitosans have degrees of acetylation between 15% and 30%. The degree of acetylation (DA) and the molecular weight of chitosan define practically all its physicochemical properties and determine its use for applications in medicine, biotechnology and other fields involving biomaterials [41].

Average degree of acetylation ($\overline{DA}$) is defined as the average percentage of remaining acetyl groups along the chain of chitin after its deacetylation. There are several methods to determine it, from simple to very sophisticated ones: elemental analysis (EA), infrared spectrometry (IR), hydrogen nuclear magnetic resonance (^{1}H-NMR), UV- spectrophotometry, linear potenciometric titration, ninhydrin test, circular dichroism measurements, others [42-43]. Among these, IR is widely used because it is relatively simple, non-destructive, cheap and generally gives reliable results. It mainly makes use of baseline (a) expressed by:

$$(\%)\overline{DA} = \left[\frac{\left(A_{1665} \big/ A_{3450} \right)}{1.33} \times 100 \right] \quad \text{— baseline (a),}$$

where $\overline{DA}$ is the degree of acetylation, i.e. the percentage of amide groups acetylated; A_{1655} and A_{3450} are the absorbance at 1655 cm^{-1} of the amide-I band as a measure of the N-acetyl group content and the hydroxyl absorbance band at 3450 cm^{-1} as an internal standard to correct film thickness or differences in chitosan concentration in powder form. This ratio of $A_{1655}/\ A_{3450}$ is used as an internal standard peak; the factor "1,33" is the value of the ratio of $A_{1655}/\ A_{3450}$ for fully N-acetylated chitosan [43,44] The degree of deacetylation, $\overline{DD}$, is then obtained by equation:

$$(\%)\overline{DD} = 100 - \overline{DA}$$

Evidently, a higher $\overline{DD}$ of chitosan indicates more free amine groups in its structure. It may contribute not only to a lowering of chitosan crystallinity up to a certain extent - crystallinity is maximum for both chitin (*i.e.* 0% deacetylated) and fully deacetylated chitosan (*i.e.* 100%) [45], but also to an improvement of its solubility in water, what is achieved by the formation of salts soluble in water after the protonation of amine groups.

Average molecular weight of chitosan is another parameter that plays an important role in determining applications for this polymer. Chen and Hwa [46] have demonstrated the influence of molecular weight of diverse chitosan samples with the same $\overline{DA}$ on its thermal, mechanical and permeability properties. Other properties which depend upon the chitosan molecular weight are antioxidant activity [47], antimicrobial activity [48], film water absorption [49], etc. Determination of chitosan molecular weight can be performed by several methods [50] usually employed in polymer chemistry: capillary viscometry [51,52], membrane osmometry [53], laser light scattering [54] exclusion size chromatography (SEC) [55] others.

Additionally, chitosan is a non-toxic, biocompatible and biodegradable linear polymer [56]. It has presented a great variety of interesting properties for cosmetic, biomedical, pharmaceutical and biotechnological applications [57]. Chemical modification of chitosan mainly aims at an improvement of its solubility in water or in other types of solvent, however it can also aim at the incorporation of special physicochemical properties to the polymer for determined applications [58]. The most common reactions of chitosan chemical modification encompass *N*-deacetylation, *N*-acetylation and *O*-acetylation [37], *N*-acylation and *O*-acylation [59-60], and *N*-phtalation [61]. Chitosan, chemically modified chitosan, and chitosan in blends with hydrophilic polymers such as poly(vinyl alcohol), poly(*N*-vinyl-2-pyrrolidone), poly(ethylene oxide), starch, cellulose [62] have raised interest in the research and development of biomaterials with promising results. However, in the last four

decades, much attention has been given to alternative methods in blending polymers to create new materials with improved physicochemical and mechanical properties for determined applications. Such methods generally are simpler and cheaper than the synthesis of new polymers. In this case, miscibility of blends is crucial in their stability and performance. Miscibility depends on polymer-polymer interactions at molecular level, mainly through H-bonds [63-64], and can be evaluated by several methods: infrared spectroscopy, differential scanning calorimetry (DSC), dynamical mechanical analysis (DMA), scanning electron microscopy (SEM), etc. [65-67,32]. Blends of chitosan and PVP can become good alternatives for producing new biomaterials, once the inherent properties of both polymers herein discussed as well as their high miscibility show very favorable performance properties for this application area.

In this work, two different techniques of hydrogel attainment were used: one from mixtures of acid aqueous co-solutions of chitosan and PVP, of which the resultant films and solutions were afterwards irradiated by ultraviolet radiation (λ = 254 nm), that is capable of inducing cross-linking of PVP by recombination of free macroradicals and subsequent hydrogel formation [25,17]; another from the reaction of poly (*N*-vinyl-2-pyrrolidone-*co*-acrolein) a novel copolymer synthesized in our biomaterials laboratory and chitosan. Cross-linking, in this case, is supposed to occur through Schiff base formation by the aldehyde group of the acrolein units of the copolymer and available amine groups of chitosan [68]. In both techniques, stable hydrogels were produced at low polymer concentrations (2% m/v, polymer/water, respectively). Some physical properties of films resulting from chitosan and PVP blends and the hydrogels from the co-solutions of both polymers as well as the hydrogels from the reaction of chitosan with the novel copolymer were characterized in order to previously evaluate their potential use as biomaterials in the near future.

2. Experimental

2.1. Materials

For the experiments, the following materials were used: two commercial chitosan samples referred to as high-molecular weight and medium-molecular weight from Aldrich (Brookfield viscosity 800.000 cP and 200.000 cP, respectively, data from the supplier), poly(*N*-vinyl-2-pyrrolidone) known as Luviskol® K-90 from BASF ($\overline{M_n}$ = 360,000; $\overline{M_w}$ = 1.200,000), acetic acid and sodium hydroxide (Vetec, São Paulo), anhydrous sodium acetate (Sigma), sodium chloride (Synth), monomers of *N*-vinyl-2-pyrrolidone and acrolein (Aldrich). Except for the polymers and monomers here cited, all reagents used in the experiments were of analytical grade.

2.2. Methods

2.2.1. Chitosan Characterization

1) *Chitosan purification*

Chitosan samples were dissolved in 2% aqueous acetic acid solution (v/v) at polymer concentration of 2% (w/v) under constant stirring overnight at room temperature. The obtained solutions were filtered through cellulose acetate MILLIPORE® membrane of 0.45 m pore size and neutralized with 10% aqueous NaOH solution (w/v) for up to three cycles. The precipitated polymer was then washed extensively with distilled water, brought to pH about 7.0 and lyophilized up to constant mass.

2) *Chitosan average degree of dacetylation* ($\overline{DD}$)

3) *Elemental analysis*

Around 20 mg of each purified chitosan in duplicate were taken for percentage determination of C, H and N in elemental analysis test carried out using a Perkin Elmer CHN analyzer. Samples were taken in duplicate.

a) *Infrared spectrometry* (*FT-IR*)

Samples of chitosan were dispersed in KBr (ca. 2% polymer, w/w) and pressed into transparent discs for analysis by FT-IR spectroscopy. Transmittance spectra were obtained through a FT-IR Bomem MB 100 spectrometer operating in a range of 350 - 4000 cm^{-1}, 2 cm^{-1} resolution, 32 runs. For DA/DD calculation, the two standard absorption bands [69] were taken into account: amide- I (1655 cm^{-1}) as a measure of the *N*-acetyl group content and hydroxyl (3450 cm^{-1} as an internal standard to correct film thickness or differences in chitosan concentration. The factor '1.33' denoted the value of the ratio of A1655/A3450 for fully *N*-acetylated chitosan.

b) *^{1}H nuclear magnetic resonance* (*^{1}H-NMR*)

^{1}H-NMR spectra were obtained on a Bruker DRX 500 spectrometer at 70˚C from chitosan solutions prepared with 10 mg of chitosan suspended in a solution composed of 1.96 mL of D_2O and 0.04 mL of DCl under constant stirring at room temperature, having 3-(trimethylsilyl)-1-propanesulfonic-d4 acid (TSPA, Aldrich) as an external reference. The calculations of DA were performed from the ratios of areas of peaks in accordance with equation

$$DA(\%) = \left(\frac{A_{CH_3}}{3A_{H2}}\right) \times 100,$$

where: A_{CH_3} = area of the peak at 2 ppm, attributed to the nuclei of the hydrogens of methyl group; A_{H2}= area of the peak at 3.2 ppm, attributed to the nucleus of hydrogen at position 2 of the glycosamine ring. By deducting from 100 the value of DA, one obtains the value of DD, that is, DD (%) = 100 – DA (%).

2.2.2. Hydrogel Attainment from Chitosan and PVPAC

1) *Preparation and characterization of poly(vinylpyrrolidone-co-acrolein) (PVPAC)*

Mixtures containing 90% of *N*-vinyl-2-pyrrolidone and 10% of acrolein (w/w) were prepared. The copolymerization was carried out in 10 mL test tubes flushed with nitrogen and duly sealed**,** using as an initiator –0.05% of azoisobutyronitrile (AIBN) by weight, based on the molar ratio of *N*-vinyl pyrrolidone/acrolein = 9. The mixtures were kept for one day at 65˚C. The obtained copolymer was purified by dissolving it in absolute ethanol and, next, precipitating it in ethyl ether. Its separation was performed by filtration. This procedure was performed up to three cycles. Afterwards, the copolymer was dried under vacuum at 60˚C to constant weight and washed with ethyl ether to remove unreacted monomer. Then, it was lyophilized for withdrawal of all remaining solvent. The percentage of the incorporated co-monomer was gotten by elemental analysis of C, H, and N on a Perkin Elmer CHN analyzer.

2) *Characterization of PVPAC*

a) *Size exclusion chromatography* (*SEC*)

The molar mass of PVPAC was evaluated in a liquid chromatograph, SHIMADZU LC-10AD equipped with refractive index and UV (254 nm) detectors (Class- LC10), utilizing Ultrahydrogel® columns (120, 250, 500 and 2000) (300 × 7,8 mm) from Waters, mobile phase of distilled water and acetonitrile (0.03 M) at a 1.0 mL/min flow. The calibration curves have been achieved with standards of poly (ethylene oxide) (PEO) with molar masses of 860,000; 348,000; 88,200; 24,800 and 1200 g·mol^{-1}.

b) *Membrane osmometry*

The number average molecular weight of PVPAC was obtained from 4 dilute aqueous solutions at different polymer concentrations in water. Osmotic pressures of diluted solutions were measured at 30˚C in a OSMOMAT osmometer, model 090, from GONOTEC (Germany), equipped with cellulose acetate membranes (cut-off 5 kDa e 10 kDa). Number average molecular weight, $\overline{M_n}$, was calculated according to equation

$$\frac{\pi}{c} = RT\left(\frac{1}{\overline{M_n}} + A_2 c\right)_{C=0}$$

where R is the universal gas constant, T temperature (K), A_2 is the second virial coefficient, $\left(\pi/c\right)_{c=0}$ is the reduced osmotic pressure extrapolated to concentration c = 0 by linear regression.

3) *Hydrogels from PVPAC and chitosan*

a) *Attainment of chitosan-PVPAC hydrogels*

The hydrogels were obtained from solutions of 80 and 120 g·L^{-1} of PVPAC copolymer and solution of 20 g·L^{-1} of chitosan dissolved in dilute aqueous acetic acid (2%, v/v). Polymer solutions were then mixed at different chitosan: PVPAC weight ratios, at pH ca. 2.5 under magnetic stirring until the attainment of a gel.

4) *Characterization of PVPAC-chitosan hydrogels*

a) *Cross-linking kinetics and mechanical properties of Schiff base hydrogel*

Cross-linking kinetics of imine hydrogels, prepared on the rheometer, between the parallel geometry plates, was performed on a cone-plate rheometer—Physica MCR 300—Paar Physica. All rheological measurements were carried out at 25˚C. The samples used in these experiments were 0.5 mL (80 and 120 g·L^{-1}) of PVPAC solution and 0.5 mL (20 g·L^{-1}) of chitosan solution.

b) Gel fraction and equilibrium swelling ratio

The hydrogel samples obtained from chitosan and PVPAC was weighed and immersed in deionized water for 48 h at room temperature. Afterwards, they were removed from the solvent, dried quickly in filter paper for withdrawal of excess water, weighed and let to dry into a ventilated oven at 70˚C during 48 h to a constant weight (w_d). The gel fraction (GF) was determined by equation:

$$(\%)GF = \frac{m_d}{m_i} \times 100$$

where % GF is the percentage of gel fraction, m_d , the mass of dry hydrogel after extraction, m_i, initial polymer mass before the extraction in deionized water.

The equilibrium swelling ratio was calculated from equation.

$$Sw_i = \frac{m_i - m_d}{m_d}$$

where Sw_i is the equilibrium swelling ratio, m_i , the mass of the swollen gel, m_d, the mass of the dry gel. For these experiments, the samples were taken in triplicate.

3. Results and Discussion

3.1. Average Degree of Deacetylation of Chitosan

The average degrees of deacetylation of both original and purified chitosans are listed in **Table 1**. They were obtained from the three different methods described herein. The three techniques utilized in determination of DD of the chitosans in our experiments generally showed results with good correlations for the original and purified samples, except for elemental analysis of the original CM, whose results were rather discrepant. The high discrepancies probably are due to presence of excess of water in its samples evaluated by this method. Chitosan is a high hygroscopic polymer in nature, and it is practically impossible to totally eliminate water contained in it by conventional drying techniques or even utilizing lyophilization. Infrared spectrometry showed to be a very efficient technique

Table 1. Average degrees of deacetylation of chitosans obtained from three different methods: elemental analysis (EA), infrared spectrometry (FTIR), and hydrogen nuclear magnetic resonance (^{1}H NMR).

	Average degree of deacetylation, $\overline{DD}$ (%)		
Chitosan	*EA*	*FT-IR*	*^{1}H -NMR*
CH (original)	60,8	63,3	63,0
CM (original)	46,4	71,0	71,0
CH (purified)	74,7	78,4	78,0
CM (purified)	76,4	77,7	78,0

in these experiments since it exhibited results very close to those from ^{1}H-NMR, considered the most reliable technique to ascertain DD of chitosan [70] for all original and purified samples. However, studies on diverse analytical methods for determination of chitosan DD [71] have demonstrated that the DD values may differ greatly when computed utilizing different baselines in the IR technique for the polymer in the forms of KBr disk and film. The IR baseline proposed by Domszy & Roberts [69] was the chosen one to compute the chitosan DDs in our work, for it has given quite satisfactory results according to literature concerned with this subject in the last three decades. Furthermore, the sample preparation and the circumstances at which it is tested may also have an influence on the results [72,73]. Despite being hygroscopic, chitosan has its water adsorption potentially diminished as its DD increases. This suggests that chitosan samples of higher DD may adsorb less moisture than those of lower DD [74]. Therefore, it is crucial that the polymer must be completely dried prior to the IR test to avoid great discrepancies in the results because even low-moisture content in samples would also contribute to the hydroxyl band that affects the DD values [75].

The ^{1}H-NMR technique for determination of chitosan DD is considered the safest one among some others introduced in literature, nevertheless it may also present dubious results concerning the peak area at the 2 ppm region attributed to the Hs of the acetamide group. For example, when DCl-D_2O is used for the polymer solubilization along the tests at 70˚C - 80˚C, effects of chitosan acid hydrolysis should be considered [76].

3.2. Copolymerization of NVP and Acrolein

The results of molar mass of the copolymer PVPAC, calculated by two different methods, are shown in **Table 2**.

In spite of the molar ratio of *N*-vinyl pyrrolidone/ acrolein = 9, this co-monomer showed to be more reactive than *N*-vinyl-pyrrolidone because its molar fraction incorporation was larger than 10 percent as expected.

As a new methodology to cross-link natural and synthetic polymers like chitosan and polymers based on *N*-vinyl-pyrrolidone, respectively, a hydrogel of chitosan

Table 2. Molar mass of copolymer PVPAC (g.mol^{-1}).

EA	Osmometry	SEC		
	$\overline{M_n}$	$\overline{M_n}$	$\overline{M_w}$	$\overline{M_w}/\overline{M_n}$
26.7 %	290,000	240,000	260,000	1.06

EA = Elemental Analysis (molar fraction incorporation)

and PVPAC was produced by the reaction of both polymers. PVP is a very hydrophilic polymer with good initial tack, transparency, chemical and biological inertness, very low toxicity as well as has high compatibility with diverse media, cross-linkable and flexible. Chitosan, in turn, is soluble only in aqueous acidic solution. As a randomly coiled cationic polyelectrolyte, it usually can be cross-linked with some dialdehydes, mainly glutarald-ehyde. The synthesized polymer, poly(*N*-vinyl-2-pyrro-lidone-*co*-acrolein) - PVPAC, reacted with chitosan, and the cross-linking occurred by Schiff base formation as shown in (**Figure 1**).

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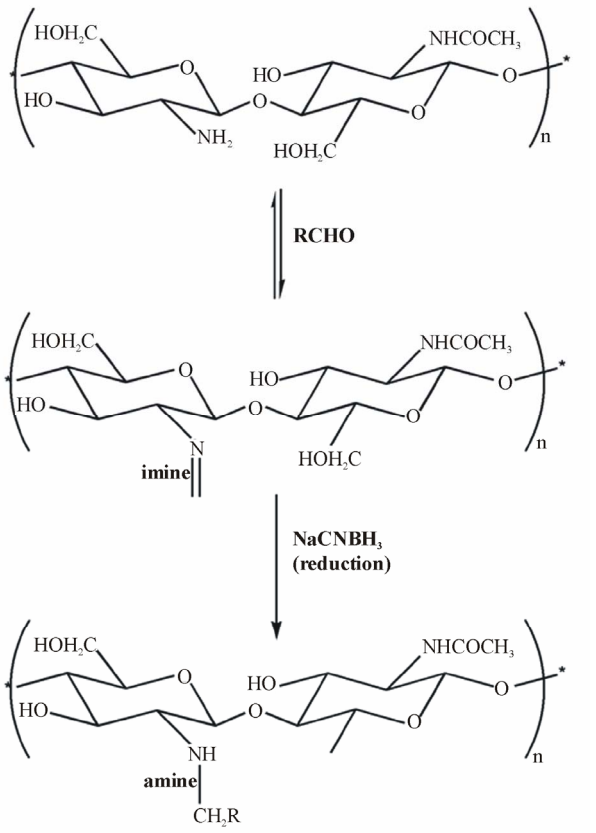


Figure 1. Schiff base formation and reduction. R = PVAC as cross-linker.

and PVPAC was produced by the reaction of both polymers. PVP is a very hydrophilic polymer with good initial tack, transparency, chemical and biological inertness, very low toxicity as well as has high compatibility with diverse media, cross-linkable and flexible. Chitosan, in turn, is soluble only in aqueous acidic solution. As a randomly coiled cationic polyelectrolyte, it usually can be cross-linked with some dialdehydes, mainly glutaraldehyde. The synthesized polymer, poly(*N*-vinyl-2-pyrro-lidone-*co*-acrolein)—PVPAC, reacted with chitosan, and the cross-linking occurred by Schiff base formation as shown in (**Figure 1**).

Throughout the experiments, PVPAC solutions were added to chitosan solutions aiming to form homogeneous hydrogels by Schiff base at ratios of 4:1, 6:1, 8:1, 12:1 (PVPAC:chitosan, w/w, respectively). The Schiff base reaction occurs between amine groups of chitosan and aldehyde functions of PVPAC, therefore contributing to formation of imine covalent linkages inter-molecularly. The polymers showed a fast cross-linking (~600 s), and their gelation kinetics were analyzed *in situ* and investigated rheologically. Correlation between gelation kinetics and hydrogel properties with PVPAC/chitosan concentration, their feed ratio, and temperature influence were also evaluated (**Figure 2**).

The chitosan–PVPAC hydrogels were then dialyzed against double-deionized water. After dialysis, transparent and elastic gels were obtained. The final products were dried in a lyophilizer until attaining constant weight. The obtained hydrogels showed a very unstable structure, *i.e*. they are very unstable mechanically, probably due to the reaction reversibility with trend to reach a balance of amine/imine in them. Cyanoborohydride is a hydride able to reduce imines to amines, and aldehydes to alcohols. We therefore proposed to produce imine hydrogels in the presence of sodium cyanoborohydride to prevent the regeneration of a non-cross-linked product. Low activity cyanoborohydride serves to narrow the competition for imine, which is formed rapidly, versus the reduction of its par-precursor. Figure 1 shows the complete chemical reaction for achieving amine hydrogels.

3.3. Infrared Spectrometry (IR)

Infrared spectra of chitosan and chitosan hydrogels (**Figure 3**) show that imine group were reduced in the presence of sodium cyanoborohydride. Most acquisitions related to chitosan are present in the imine hydrogel. The for imine, which is formed rapidly, versus the reduction of its par-precursor. Figure 1 shows the complete chemical reaction for achieving amine hydrogels. region at ~ 3500 cm^{-1} is related to the absorption of axial stretching and OH stretching bands, NH overlapping; regions from ~ 1600 to ~ 900 cm^{-1} are related to the absorption

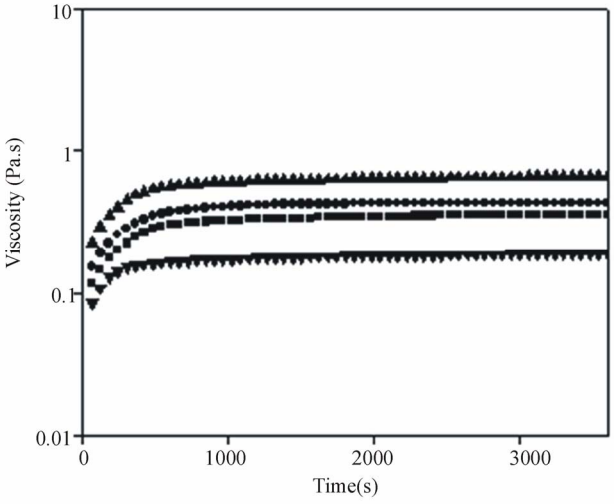


Figure 2. Kinetic curves from chitosan solution 20 g·L^{-1}: ▲CH and PVPAC 120 g·L^{-1}; ● CH and PVPAC 80 g·L^{-1}; ■ CM and PVPAC 120 g·L^{-1}; ▼ CM and PVPAC 80 g·L^{-1}.

owing to deflection angle of NH, the deformation of symmetrical angular CH_3 of amide, C-N stretching of amide and C-N stretching of amine. Those with acquisition number of frequencies smaller than 900 cm^{-1} indicate bands related to polysaccharide structures. An absorption band very close to 1575 cm^{-1} in the spectrum suggests the stretching band of C = N linkage present in the hydrogel.

The spectrum of the amine hydrogel (imine reduced by cyanoborohydride) shows a decrease in the $\nu_{C=N}$ intensity of the stretching of the linkage C = N. Thus, there is an increase in the intensity of NH deflection angle of the band produced by amine.

According to **Figure 5(a)**, imine hydrogels lose viscosity as shear rate increases. Such hydrogel sets flow by shear ("shear-thinning"). This behavior is typical of pseudoplastic materials. This is an interesting property of materials known as "smart materials", which can be exploited in various biomedical applications. It has been presumed that the rheological behavior of these hydrogels might be attributed to the disruption of the imine balance in crosslinked hydrogel/aldehyde (non-crosslinked) by mechanical forces. However, even in the presence of $NaCNBH_3$ (**Figure 5(b)**), this behavior remains, which suggests that such behavior is not provoked by the balance shift of imine/aldehyde in imine hydrogels, but more probably by irreversible breakages of their three-dimensional networks.

Due to the fast kinetics and some interesting physical properties of these hydrogels, their gel fractions and swelling ratios were evaluated. **Table 2** exhibits the results obtained.

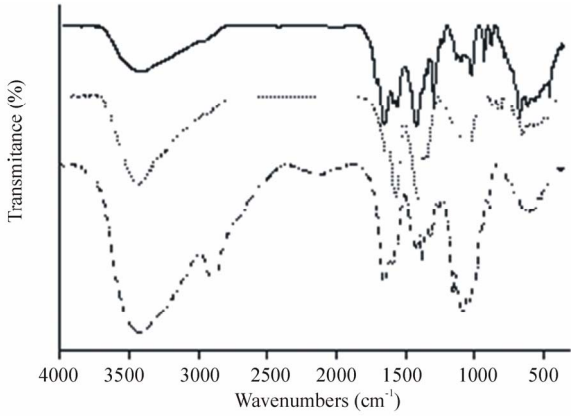
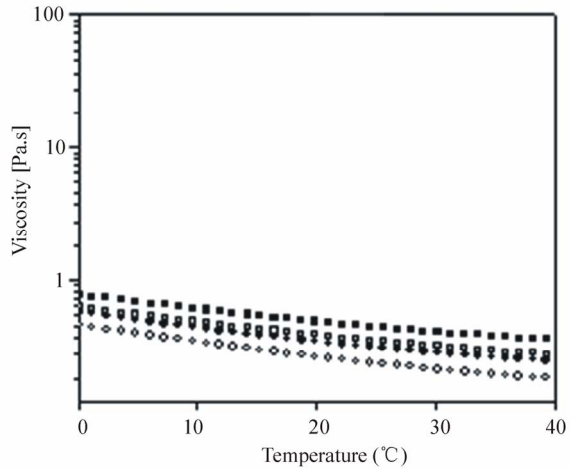
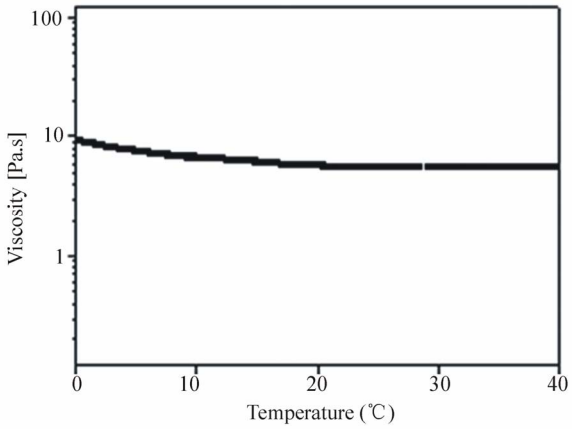


Figure 3. IR spectra of dry chitosan sample (_ _), Schiff base hydrogel (.....) and reduced Schiff base hydrogel (___).



(a)



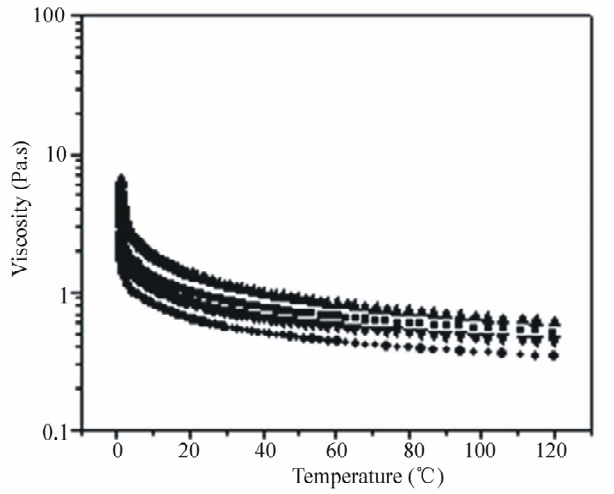
(b)

Figure 4. Viscometric analysis at different temperatures (a) chitosan solution 20 g·L^{-1}: ■ CH and PVPAC 120 g·L^{-1}; □ CH and PVPAC 80 g·L^{-1}; ● CM and PVPAC 120 g·L^{-1}; ○ CM and PVPAC 80 g·L^{-1} and (b) [PVPAC] = 80 g·L^{-1}, [CM] = 20 g·L^{-1} and [NaCNBH$_3$] = 20 g·L^{-1}.

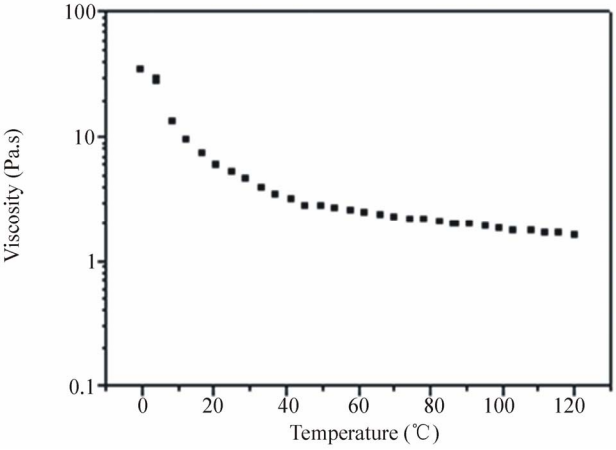
3.4. Gel Fraction and Equilibrium Swelling Ratio of Chitosan-PVPAC

Table 3 shows that gel fractions obtained from imine hydrogels have high values, and the concentration of copolymer PVPAC does not interfere in gel fraction results. These high gel content values show a good portion of polymer that did not dissolve in solvent due to cross-linking between PVPAC and chitosan, *i.e.* high content of chitosan and PVPAC reacted.

Swelling ratio is highly dependent upon the concentration of copolymer PVPAC. It seems that PVPAC acts as a chitosan cross-linker because, at higher concentrations, there is more capacity of water absorption in the hy-



(a)



(b)

Figure 5. Shear rate (a) chitosan solution 20 g·L^{-1} ▲ CH and PVPAC 120 g·L^{-1}; ■ CH and PVPAC 80 g·L^{-1}; ● CM and PVPAC 120 g·L^{-1}; ▼ CH and PVPAC 80 g·L^{-1}. (b) [PVPAC] = 80 g·L^{-1}, [CM] = 20 g·L^{-1} and [NaCNBH$_3$] = 20 g·L^{-1}.

Table 3. Average gel fractions and equilibrium swelling ratios of hydrogels obtained from mixtures of chitosan and PVPAC solutions in diverse proportions. Samples taken in triplicate.

Samples	*CM*		*Samples*	*CH*	
	Average gel fraction (%)	*Average swelling ratio*		*Average gel fraction (%)*	*Average swelling ratio*
4:1	86 ± 15	31 ± 9	4:1	82 ± 2	76 ± 9
6:1	87 ± 10	25 ± 9	6:1	93 ± 4	49 ± 15
8:1	85 ± 9	32 ± 11	8:1	50 ± 6	44 ± 9
12:1	94 ± 3	30 ± 6	12:1	92 ± 3	31 ± 10

Table 4. Average gel fractions and equilibrium swelling ratios of hydrogels obtained directly from chitosan-PVP blend films and mixtures of chitosan and PVP co-solutions after submitted to $UV_{254\ nm}$ irradiation for 1.5 h (films) and 4 h (solutions) at ca. 30˚C, under N_2 atmosphere. Samples in triplicate.

Samples	Films		Samples	Hydrogel*	
	Average gel fraction (%)*	Average equilibrium swelling ratio*		Average gel fraction (%)**	Average equilibrium swelling ratio ***
PVP	64 ± 6	97 ± 21	PVPac	63 ± 8	250 ± 50
CM5	74 ± 16	45 ± 8	CM5	76 ± 4	240 ± 30
CH5	80 ± 16	79 ± 4	CH5	88 ± 3	160 ± 30
CM50	79 ± 2	27 ± 5	CM20	70 ± 5	280 ± 30
CH50	88 ± 2	30 ± 3	CH20	51 ± 7	430 ± 120
CM95	93 ± 7	28 ± 8	CM30	26 ± 8	740 ± 210
CH95	94 ± 5	30 ± 2	CH30	41 ± 7	490 ± 120

PVPac: PVP dissolved in aqueous acid solution : 2 g of PVP : 98 g of 2% acetic acid-H_2O (v/v) Numbers on the right of CM and CH represents weight % chitosan in dry hydrogel. * Films immersed in deionized water for 48 h at room conditions; ** After 24 h of Sohxlet extraction in deionized water, dried for 72 h at 70˚C in ventilated oven; *** After Sohxlet extraction, hydrogels kept immersed in deionized water for 48 h at room conditions.

drogel probably influenced by the formed networks allied to the high hydrophilicity of the former. On the other side, the hydrogel produced with chitosan of medium molecular weight (CM), differently of that with chitosan of high molecular weight (CH), didn't show dependence on PVPAC concentration in the swelling test. That can be related to the fact that the swelling limit had already reached the maximum with CM.

The results obtained for the Schiff base hydrogels were compared with those of hydrogels attained from films of chitosan–PVP blends prepared by casting on plastic molds as well as mixtures of 2% polymer co-solutions at different polymer ratios on a dry basis, packed and sealed in quartz tubes. The samples in triplicate were submitted to the same UV radiation dose and reaction time in a UV chamber with an N_2 atmosphere. **Table 3** gives a summary of the effect of the formulation compositions on average gel fractions and swelling ratios. These hydrogels are likely semi-interpenetrating networks (SIPNs), capable of keeping chitosan and PVP non-cross-linked molecules entrapped within the networks, increasing hence the gel fractions. Their values also corroborate the assumption of the existence of a strong interaction between both polymers, which form miscible blends [77,78], as well as the formation of probable SIPNs induced by UV cross-linking, in which the cross-linked part is probably constituted of PVP. Attempts to cross-link films or solutions made of pure chitosan without any photoinitiator or chemical modification of this polymer haven't afforded convincing results with this process in our experiments under the conditions described here.

It has been verified that, in attainment of hydrogels from mixtures of 2% polymer co-solutions, the gel fractions increase as PVP content increases in the mixtures, confirming, therefore, that this polymer effectively is more prone to cross-link than chitosan in these experiments. Nevertheless, there is a limitation on directly obtaining hydrogels from mixtures of diluted acid aqueous solutions of chitosan and PVP after being irradiated by UV light, when such mixtures contain amounts above 30% of chitosan (w/w) on a dry basis. This fact may be owing to a similar reactivity observed by Zhao *et al.* [79] in attainment of hydrogels from blend of carboxymethyl-chitosan and PVP using EB-radiation. Hence, chitosan oligomers and other likely products derived from its degradation by UV irradiation would be acting as scavengers of free radicals and would interfere with the cross-linking of PVP, leading to products insufficiently cross-linked to form hydrogels.

3.5. Gel Fractions and Swelling Ratios of Chitosan-PVP Mixtures

See **Table 4**

4. Conclusions

4.1. Hydrogels from Poly(N-vinyl-2-co-Acrolein) and Chitosan Reaction

The novel copolymer of *N*-vinyl-2-pyrrolidone and ac-

rolein reacted with chitosan and produced a hydrogel through imine bonds. A reduction of imine to amines with sodium cyanoborohydride has shown to be a good alternative to stabilize the structure of the hydrogel. It was demonstrated that the dynamic equilibrium linkage between the polymers can be a controllable parameter as well as an interesting property that can be exploited in further experiments. Moreover, the hydrogel has a potential uses as wound dressings and systems for controlled release of drugs, because of the compatibility of PVP with the body fluids, added to the properties of the chitosan as linker and binder [80-82], fungicide, and it has a good permeability to gas, immunogenic compatibility, low toxicity and good bioabsorption. Attainment of hydrogels from Schiff base by reaction of poly(*N*-vinyl-2-*co*-acrolein) and chitosan proved to be an effective methodology for the production of hydrogels. The resultant reaction products exhibit high values of gel content and swelling ratio, and provide a material that maintains some characteristics of both hydrogels from PVP and chitosan. Another advantage of this process is that they may also be produced from more concentrated solutions of initial polymers.

4.2. Hydrogels from Mixtures of Aqueous Acidic Co-Solutions of Chitosan and PVP

In relation to the hydrogels obtained from chitosan-PVP films or directly from mixtures of aqueous acidic co-solutions of both polymers in diverse proportions under UV radiation for inducing cross-linking, their resultant gel fractions and swelling ratios have also shown that these hydrogels are promising materials for several applications, for example, as biomaterials.

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