Poly(*N*-vinyl-2-pyrrolidone) hydrogels produced by Fenton reaction

J.A.G. Barros, G.J.M. Fechine, M.R. Alcantara, L.H. Catalani*

Instituto de Química — Universidade de São Paulo, Av. Prof. Lineu Prestes 748, CP 26077 CEP 05508-900, São Paulo, SP, Brazil

Abstract

'Instantaneous' hydrogels from poly(*N*-vinyl-2-pyrrolidone) aqueous solutions can be produced using Fenton reagents, *i.e.*, a redox reaction. A firm hydrogel is formed within 5–10 s, with a total gelling time below 8 min. Hydroxyl radical is recognized as the possible high energy intermediate responsible for the crosslinking reactions. The major variables of the process (PVP, H_2O_2 and Fe^{2+} initial concentrations, as well as pH) were examined and shown to operate within a rather narrow working window. The process efficiency and the properties of the hydrogel formed are discussed and compared with other methods of hydrogel production.
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Keywords: Hydrogel; Fenton reaction; Instantaneous gel

1. Introduction

Hydrogels are polymeric networks, which absorb and retain large amounts of water. The steps of hydrogel formation essentially involve (i) monomer polymerization in the presence of various amounts of a bifunctional comonomer or (ii) crosslinking of preformed polymers [1]. Hydroxyl radicals, a highly reactive species, have been used as initiators for crosslinking reactions. In the presence of polymer macromolecules bearing labile hydrogen atoms they react to produce macroradicals. If favorably positioned, these radicals may undergo recombination leading to a covalent crosslink. For this purpose, hydroxyl radicals have been produced by water radiolysis [2,3] or by alternative methods that use UV radiation and hydrogen peroxide [4].

The Fenton reaction is a process that relies on the reduction of hydrogen peroxide at the expense of Fe^{2+} ions [5–7]. In spite of being known for more than a century, this complex system is still fierce debate over which true oxidative species is active [8]. The dispute is between a free radical mechanism, where hydroxyl radical is primarily formed, and an ionic mechanism involving an oxoiron(IV) species (FeO^{2+}). A great number of studies have investigated the use of Fenton reactions for water remediation through pollutant degradation [9–11]. Generally, it is assumed that the radical mechanism is operative [5–7].

Despite the dispute, if hydroxyl radicals are generated by Fenton reactions they are expected to have the same behavior as the hydroxyl radicals produced by high-energy radiation or by photolysis of H_2O_2. Fenton reagents have been used as radical initiator in vinylic polymerization or grafting for more than 50 years [12–15]. However, almost no reference has been made to its use to initiate crosslinking [16].

In this work we evaluate the crosslinking process of the PVP aqueous solution by Fenton reactions. The fast kinetics observed for Fenton reactions is anticipated to be a major advantage. The optimization of the major parameters of the process (PVP concentration, pH and $[H_2O_2]/[Fe^{2+}]$ ratio) was carried out by using a 2^3 factorial experimental design which evaluates three factors and two levels.

* Corresponding author. Tel.: +55 11 3091 3162; fax: +55 11 3815 5579.
E-mail addresses: janabar@iq.usp.br (J.A.G. Barros), fechine@iq.usp.br (G.J.M. Fechine), mralcant@iq.usp.br (M.R. Alcantara), catalani@usp.br (L.H. Catalani).

2. Experimental

2.1. Materials and methods

The PVP used, known as Luviskol® K-90 ($M_w = 1.2 \times 10^6$) was supplied by BASF. The hydrogels were produced by mixing two solutions: solution A containing PVP and hydrogen peroxide (Merck 30% stock solution) and solution B containing PVP (same concentration as solution A) and ferrous chloride (Aldrich), at room temperature. Concentration of H_2O_2 was set as 10 mM or 100 mM and that of $FeCl_2$ as 5 mM or 10 mM, such that the ratio $[H_2O_2]/[Fe^{2+}]$ was 1 and 10. Moreover, to evaluate the pH influence, PVP solutions were also prepared in buffer solutions at pHs 2.5 (acetate 0.1 M) and 7.0 (phosphate 0.1 M). The H_2O_2 stock concentration was measured by UV absorption as described in Ref. [17] using a Multispec 1501 – Shimadzu spectrometer.

The gel content, $g(\%)$, swelling ratio, (Q), crosslink density, ρ_x, and mesh size, ξ, were calculated as described elsewhere [18]. Briefly, the gel content of the samples was calculated according to Eq. (2), after Soxhlet extraction with water (36 h) and drying to constant weight (60 °C for 24 h):

$$s(\%) = \left(\frac{w_g - w_0}{w_0} \right) \times 100 \tag{1}$$

$$g(\%) = 100 - s(\%) \tag{2}$$

where $s(\%) =$ sol content; $g(\%) =$ gel content; $w_g =$ weight of PVP used to prepare hydrogel; $w_0 =$ weight of dried gel.

The swelling ratio (Q) was calculated according to Eq. (3), after the sample reached maximum swelling by immersing it in distilled water at 25 °C for 12 h:

$$Q = \frac{w_s - w_d}{w_d} \tag{3}$$

where w_s is the weight of swollen hydrogel and w_d is the weight of dried hydrogel.

The crosslink density ρ_x was calculated according to Eq. (4) [see Ref. [19]]:

$$\rho_x = \frac{1}{\overline{\nu} M_c} = \frac{2}{\overline{\nu} \overline{M}_n} + \frac{(1/V_1) \left[\ln(1 - V_{2s}) + V_{2s} + \chi_1 V_{2s}^2 \right]}{V_{2r} \left[(V_{2s}/V_{2r})^{1/3} - 1/2(V_{2s}/V_{2r}) \right]} \tag{4}$$

where $\overline{M}_n$ is the number-average molecular mass (3.6×10^5 g/mol for the PVP used), V_{2s} is the polymer volume fraction in the swollen gel at equilibrium, V_{2r} is the polymer volume fraction in the relaxed state, V_1 is the molar volume of the solvent, $\overline{\nu}$ is the polymer specific volume (the reciprocal of the polymer density; 0.785 cm³/g for PVP) and χ_1 is the solubility interaction parameter for PVP–water system.

The mesh size ξ (in nm) of the polymer network was calculated by Eq. (5) [see Ref. [20]]:

$$\xi = 0.1 V_{2s}^{-1/3} \left(C_n \frac{2M_c}{M} \right)^{1/2} \delta \tag{5}$$

where M is the monomeric unit molecular mass, C_n is the rigidity coefficient, and δ is the length of an individual C–C bond (0.154 nm). The value of 6.9 [20] for poly(methylmethacrylate) was used as C_n.

The viscosity measurements were performed on a plate/plate MCR 300 – Paar Physica rheometer. All viscosity measurements were carried out at 25 °C. Fenton reaction kinetics was followed by measuring the increase in viscosity in real time, disregarding the first 60 s for mixing. The sample was prepared by mixing *in situ* equal volumes of solutions A and B.

The cytotoxicity of the membranes was tested by an *in vitro* cell viability method according to Ciapetti et al. [21]. The test was carried out with dilution of the extracts of hydrogel membranes, in contact with mouse subconjuntive tissue cell culture from NCTC Clone 929 line (ATCC-CCL1). Phenol solution (0.02%) and poly(vinyl chloride) (PVC) extracts were used as positive and negative controls, respectively.

2.2. Factorial experiment design

To evaluate the effects of polymer concentration, Fenton reagents ([H_2O_2] to [Fe^{2+}] ratio) and pH upon hydrogel production, a 2^3 factorial experimental design with 3 replications in a total of 24 runs was employed. Statistical data treatment was performed by Statistica 5.0 software (StatSoft Inc.) and was based on the analysis of variance (ANOVA).

ANOVA table produces relevant values like the mean squares (MS) (Eq. (7)) of the independent variables, their interaction and residual. MS values were calculated from the sum of squares (SS) (Eq. (6)) divided by the degrees of freedom (df). *F-test* (square of effects/squares of residual) and *p-value* (significance probability) (Eq. (8)) were calculated in order to evaluate the significance effects. The *p-value* represents the probability to obtain a specific value in the F distribution with $k - 1$ variables and $k(n - 1)$df higher or equal to F_0 (k is the population number and n is the sample size). With the *p-value*, it is possible to evaluate if the dependent variables are significant, how significant, and their interaction. The significance level (α) for the global statistical treatment was set to 0.05, which is typically used as standard value for 2^3 factorial experiments collected in triplicates [22–24]. From these values and degrees of freedom it is possible to obtain F_0 value, from a reference table. If the calculated F value is higher than the F_0 value, the represented effect is considered statistically relevant. The independent variable intervals used in this work are shown in Table 1. The dependent variables are gel content, crosslink density, swelling ratio and mesh size. They were selected based on a previous study of PVP hydrogels produced by UV radiation [4]. Surface response

Table 1
Factor levels of the independent variables for the production of PVP hydrogels by Fenton reaction

Independent variable	Label	Low (−1)	High (+1)
PVP concentration (g/L)	x	80	120
[H_2O_2]/[Fe^{2+}]	y	1	10
pH	z	2.5	7.0

equations are expressed only in terms of relevant dependent variables.

$$SS = \sum_{i=1}^{n} \sum_{j=1}^{n} \left(x_{ij} - 1/nk \sum_{i=1}^{n} \sum_{j=1}^{n} x_{ij} \right)^2 \qquad (6)$$

$$MS = SS/df \qquad (7)$$

$$p\text{-}value = P[F(k-1), k(n-1) > F_0] \qquad (8)$$

Different values of $[H_2O_2]$, $[Fe^{2+}]$ and [PVP] other than the ones reported have been investigated, however, the results of gel content were not satisfactory. A fine equilibrium of hydroxyl radical formation is necessary to avoid degradation reactions.

Three-dimensional plots were drawn using SigmaPlot 8.0 (Systat Software Inc.).

3. Results and discussion

Hydroxyl radical is the most reactive radical among the reactive oxygen species (ROS). Its reaction with PVP is expected to generate macroradicals centered in three possible positions, taking into account the lability of the hydrogen atoms present in its structure (see Scheme 1). It is generally accepted that the rate of hydrogen abstraction is dependent on the dissociation energy of the X−H bond to form the radical. Basic thermodynamic calculations show that C−H bonds α-positioned to a heteroatom or a carbonyl are lower in energy, mainly due to the stabilization of the radical product. From the 5 possible C−H bonds in PVP structure, two of them do not fall into this category and are considered much less reactive. Recombination reactions following its generation lead to crosslink formation.

The bimolecular reaction of Fe^{2+} and H_2O_2 presents a relatively fast kinetics, with a rate constant of the order of 50–70 $M^{-1} s^{-1}$ [6,25]. Therefore, under the conditions used, where H_2O_2 is either 50 or 100 mmol/L, 95% of the initial Fe(II) is oxidized within 2–4 s. The Fe^{2+} formed is regenerated to Fe^{3+}, by reaction with H_2O_2, but at a much slower rate. If hydroxyl radical is indeed formed from this primary bimolecular reaction, its immediate initial increase in concentration would lead to hydrogel formation.
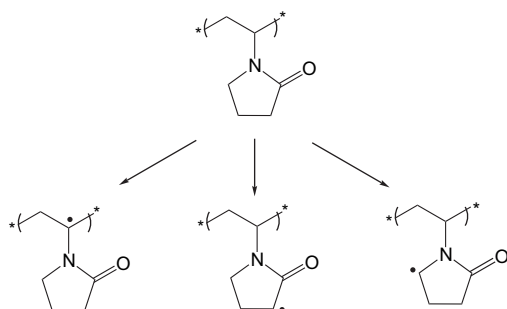
It was observed that when the two PVP solutions, one containing H_2O_2 and another containing $FeCl_2$ are mixed, a consistent hydrogel is obtained almost instantly, within 5–10 s. Several attempts to examine the initial kinetics of this process have failed. Given the short observed time span of this initial process, a flow technique would be required; however, the high viscosity of the primary solutions precludes this approach.

It was also noted that, after the mixing, the total gelling time is longer, up to a few minutes. Hence, the kinetics of this slow phase and total gelling time was measured by rotational rheometry. The dynamic viscosity versus time profile of the second phase showed that (i) there is a drastic increase in the viscosity (~25 times) within the first minute, where most of the gel is formed and (ii) the kinetics of the second phase is similar regardless of how the parameters were varied. Fig. 1 shows the viscosity profile of the reaction starting at 60 s from mixing (the time required for starting measurement).

For the most important anticipated applications, the significant properties and dependent variables of the hydrogels produced are (i) gel content, (ii) swelling ratio, (iii) mesh size and (iv) crosslink density. These properties represent the statistical responses that were evaluated against the key independent variables of the process: (i) the polymer concentration, (ii) pH and (iii) relative concentrations of the Fenton reagents (H_2O_2 and Fe^{2+}). For optimization purposes a 2^3 factorial experimental design was employed.

Table 2 lists the raw data of the responses obtained for each combination of factors where the high and low values for each factor are coded as +1 and −1, respectively. Table 3 shows the main effects of independent variables and their respective combinations, and Table 4 shows the significance level of all dependent variables through F-test and p-values. If the F value for a given effect is higher than the F_0 (F-theoretical) this effect is significant. An independent variable has a significant influence whenever the p-value is lower than 0.05. The main effect shown in Table 3 represents the improvement obtained
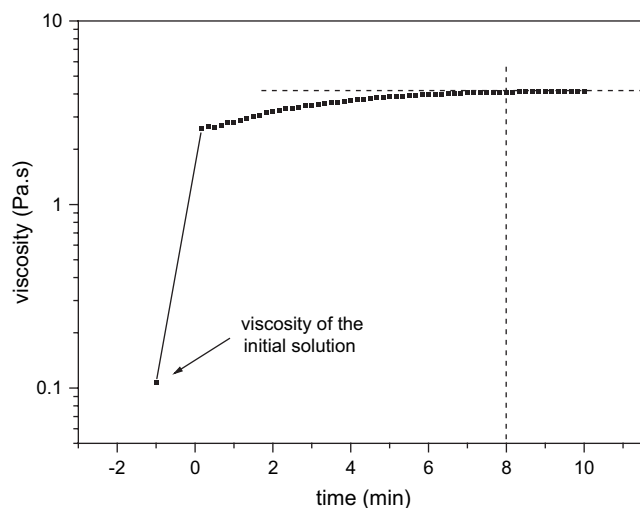
Scheme 1.

Fig. 1. Dynamic viscosity profile of an 8% PVP aqueous solution subjected to Fenton reagent ($[FeCl_2] = 5$ mmol/L; $[H_2O_2] = 50$ mmol/L; acetate buffer = 0.1 mol/L at pH 2.5).

Table 2
Crude results for all limits of the independent variables

Variable limits[a] x, y, z	Gel content (%)	Swelling	Crosslink density (10^{-6} mol/cm^3)	Mesh size (nm)
+1, +1, +1	68 ± 3	15 ± 4	11 ± 1	6.0 ± 0.1
+1, +1, −1	64 ± 2	15 ± 1	10.6 ± 0.3	5.7 ± 0.2
+1, −1, +1	67 ± 2	24 ± 1	8.8 ± 0.2	6.9 ± 0.2
+1, −1, −1	61 ± 1	27 ± 2	8.5 ± 0.2	7.3 ± 0.2
−1, +1, +1	77.9 ± 0.3	32 ± 2	8.1 ± 0.1	7.7 ± 0.1
−1, +1, −1	93 ± 4	23.4 ± 0.6	8.8 ± 0.1	6.9 ± 0.1
−1, −1, +1	63 ± 5	27.6 ± 0.6	8.32 ± 0.05	7.4 ± 0.1
−1, −1, −1	88 ± 1	17 ± 1	9.9 ± 0.2	6.1 ± 0.1

[a] See Table 1 for label value and limit value.

Table 3
Results for main effects of all independent variables and its interactions

	Main effects			
	Gel content	Swelling	Crosslink density ($\times 10^{-7}$)	Mesh size
[PVP] (x)	−15.37	−4.91	9.5	−0.53
[H$_2$O$_2$]/[Fe^{2+}] (y)	5.67	−2.74	7.4	−0.33
pH (z)	−7.57	3.80	−0.4	0.49
$x−y$	−3.74	−7.78	14.0	−0.91
$x−z$	12.21	−5.43	7.4	−0.54
$y−z$	2.12	0.35	2.1	0.04
$x−y−z$	−3.09	1.50	−0.2	0.28

by changing the factor level of the independent variables from low to high. While the sign of the effect indicates an increase (when positive) or decrease (when negative) of the parameter; its absolute value indicates the total change observed.

As seen in Tables 3 and 4, [PVP] is the most important parameter in determining gel content. This statement is based on the higher value of [PVP] effect combined with lower *p-value* (equal to zero), together with the highest F value. Gel content measures the inclusion of the macromolecules into the

Table 4
F-test[a] and significance probability (*p-value*) for all dependent variables: gel content, swelling, crosslink density and mesh size

	F-estimated (*p-value*)			
	Gel content	Swelling	Crosslink density	Mesh size
[PVP] (x)	221.01 (8.74×10^{-11})	48.40 (3.23×10^{-6})	13.20 (2.23×10^{-3})	110.84 (1.34×10^{-8})
[H$_2$O$_2$]/[Fe^{2+}] (y)	30.11 (4.97×10^{-5})	15.07 (1.32×10^{-3})	7.85 (1.28×10^{-2})	41.83 (7.77×10^{-6})
pH (z)	53.65 (1.71×10^{-6})	29.11 (5.95×10^{-5})	*2.38* (*0.142193*)	91.75 (4.98×10^{-8})
$x−y$	13.09 (2.31×10^{-3})	121.60 (6.93×10^{-9})	29.78 (5.30×10^{-5})	317.94 (5.57×10^{-12})
$x−z$	139.35 (2.61×10^{-9})	59.20 (9.12×10^{-7})	7.92 (1.25×10^{-2})	112.92 (1.17×10^{-8})
$y−z$	*4.22* (5.66×10^{-2})	*0.26* (6.20×10^{-1})	*0.68* (4.21×10^{-1})	*0.49* (4.91×10^{-1})
$x−y−z$	8.94 (8.67×10^{-3})	4.56 (4.83×10^{-2})	*0.70* (4.14×10^{-1})	30.45 (4.70×10^{-5})

[a] F-theoretical (F_0) = 4.49 for $\alpha = 0.05$ and degrees of freedom (df) total = 16.

Scheme 2.

reticulate through crosslinking. On the other hand, crosslinking depends on diffusion of macroradicals formed towards each other. Since this is a limited process, it is expected that concentration of the polymer to ultimately control this parameter.

The equations from Scheme 2 suggest that the presence of H$^+$ is required for the decomposition of H$_2$O$_2$, indicating the necessity of the acid environment to produce the maximum amount of hydroxyl radicals in a Fenton system [5]. The pH effect upon this redox crosslinking system is reflected in every parameter measured, as shown in Fig. 2. Additionally, pH seems to play a major role since its *p-value* alone or in conjunction with [PVP] ($x−z$ term) is very small, therefore, significant. Fig. 2A shows how [PVP], [H$_2$O$_2$]/[Fe^{2+}] ratio and pH influence the gel content. It also shows that the highest value of gel content takes place when [PVP] is equal to 80 g/L, pH = 2.5 and [H$_2$O$_2$]/[Fe^{2+}] = 10.

For the swelling behavior of PVP hydrogels obtained by Fenton reactions, the most significant independent variable is the interaction between [PVP] and [H$_2$O$_2$]/[Fe^{2+}] ratio, as seen in Table 3. These data are confirmed by *p-value* and F-test from Table 4. On the other hand, [H$_2$O$_2$]/[Fe^{2+}] ratio and pH interaction (*yz*) were not relevant at a significant level of 5%. The swelling behavior is correlated with the water absorption capacity by the hydrogel. In fact, crosslink density is the main factor that controls this capacity.

The crosslink density depends on parameters like available hydroxyl radical, medium pH and [PVP]. The most significant term for crosslink density shown in Tables 3 and 4 is the interaction between [PVP] and [H$_2$O$_2$]/[Fe^{2+}] ratio. However, pH (z) and its interactions *yz* and *xyz* were not relevant at a significant level of 5%. The Fig. 2C shows the surface response for crosslink density as a function of [PVP], [H$_2$O$_2$]/[Fe^{2+}] ratio and pH. The surface response shows that higher values of the crosslink density are obtained when [PVP] and [H$_2$O$_2$]/[Fe^{2+}] ratio are at maximum level. It means that polymer networks are more compact, with lower swelling capacity. In other words, hydrogels that show a great swelling have a higher water absorption capacity due to lower crosslink density.

Mesh size dependence can be accessed through Fig. 2D, as a function of the [PVP], [H$_2$O$_2$]/[Fe^{2+}] ratio and pH. According to Tables 3 and 4, [PVP], its interaction with [H$_2$O$_2$]/[Fe^{2+}] and pH have strong influence on mesh size. As can be seen, the lower values of the mesh size coincide with the region of the higher values of the crosslink density, consequently, lower values of the swelling. This region is localized when [PVP] and [H$_2$O$_2$]/[Fe^{2+}] are at maximum level or at minimum for a pH equal to 2.5. The behavior at pH = 7.0 is
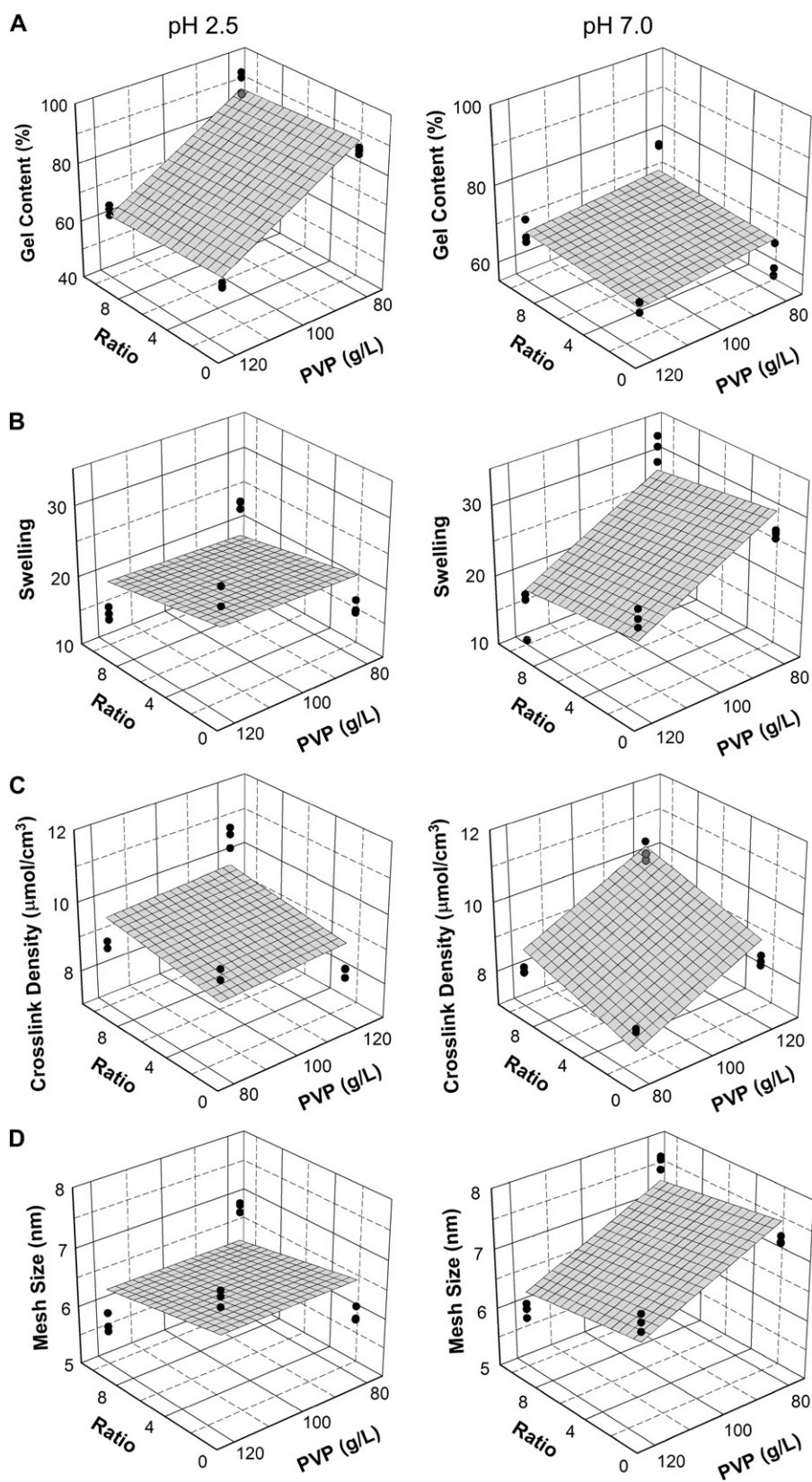
Fig. 2. Surface graphs of product parameters as a function of the [PVP] and [H$_2$O$_2$]/[Fe^{2+}] ratio and pH. (A) Gel content; (B) swelling; (C) crosslink density; (D) mesh size; all indexes 1 (left) at pH 2.5 (acetate buffer 0.1 mol/L); all indexes 2 (right) at pH 7.0 (phosphate buffer 0.1 mol/L).

Table 5
Comparison of PVP hydrogel produced by different methods

Methods	Gel content (%)	Swelling	Crosslink density (10^{-6} mol/cm^3)	Mesh size (nm)
Ionizing radiation[a]	79 ± 1	—	2.00	92
Direct UV[b]	82 ± 2	40 ± 3	8.30 ± 0.05	7.39 ± 0.05
Sensitized UV[c]	81 ± 2	37 ± 4	8.8 ± 0.2	6.9 ± 0.2
Photo-Fenton[d]	80 ± 3	—	9.0 ± 0.4	6.7 ± 0.3
Fenton (this work)[e]	93 ± 4	23.4 ± 0.6	8.8 ± 0.1	6.9 ± 0.1

[a] Ref. [26]: [PVP] = 80 g/L, [agar] = 8 g/L, [PEG] = 30 g/L, dose = 20 kGy (electron-beam).
[b] Ref. [4]: [PVP] = 80 g/L, dose = 1814 J (254 nm radiation).
[c] Ref. [4]: [PVP] = 80 g/L, [H_2O_2] = 10 mmol/L, dose = 304 J (254 nm radiation).
[d] Ref. [4]: [PVP] = 80 g/L, [H_2O_2] = 50 mmol/L, [Fe^{3+}] = 5 mmol/L, dose = 853 J (360 nm radiation).
[e] This work: [PVP] = 80 g/L, [H_2O_2] = 50 mmol/L, [Fe^{2+}] = 5 mmol/L, pH = 2.5.

similar only in maximum level, probably because Fenton reactions do not follow the same kinetics pattern at this pH.

Other methods to crosslink PVP include the use of high-energy radiation [2,3], direct UV [26], sensitized UV and photo-Fenton [4]. All these methods give hydrogels with very similar characteristics. Table 5 shows a comparison of g, Q, ρ_x and ξ values.

The cytotoxicity tests showed that hydrogels prepared by these methods do not impose any toxic effect. These hydrogels were also submitted to dermal inflammation test in albino rabbits, where dressings made of hydrogel were maintained in direct contact with the rabbit skin for 72 h. All samples presented inflammation indexes within a satisfactory range, *i.e.*, as a non-irritating material.

4. Conclusions

A hydrogel can be produced by crosslinking a PVP aqueous solution using Fenton reagents, *i.e.*, through a redox system. Hydroxyl radical is the 'high energy' intermediate most likely responsible for macroradical formation, followed by recombination reactions. The main variables controlling the process are PVP concentration, pH and the ratio [H_2O_2]/[Fe^{2+}]. Optimum conditions for gel content and crosslink density were determined through a 2^3 factorial experiment design as [PVP] being equal to 80 g/L, pH = 2.5 and [H_2O_2]/[Fe^{2+}] = 10.

The important aspect of this process is the possibility of 'instantaneous' production of hydrogels, with a consistent product formed within 5–10 s. We believe this is an important feature of the process that will meet the requirements of a broad range of applications in the field.

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