Crosslinking of poly(vinylpyrrolidone) activated by electrogenerated hydroxyl radicals:

A first step towards a simple and cheap synthetic route of nanogel vectors

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Abstract

A facile electrosynthesis route for the preparation of polymer nanogels based on the in situ production of hydroxyl radicals is reported for the first time. Electro-Fenton process with continuous H_2O_2 electrogeneration and Fe^{2+} regeneration performs better than electro-oxidation with a boron-doped diamond or dimensionally stable anode for promoting crosslinking of poly(vinylpyrrolidone).

1. Introduction

Polymer nanogels have been proposed as nanovectors to deliver drug molecules in living tissues under targeted and controlled release conditions [1,2]. They can be fabricated by surfactant-assisted polymerization of hydrophilic monomers in the presence of crosslinking agents but, toxicity of residual monomer, initiator and surfactant may impede their use in biomedicine [3]. A clean approach to prepare high purity nanogels is based on high energy irradiation of aqueous solutions of preformed polymers to generate free radicals onto the macromolecules [4,5]. Nanogels can be obtained by enhancing intramolecular combination of active centres using dilute polymer solutions exposed to ionizing radiation at relatively high doses per pulse [4]. Nanogels of poly(acrylicacid), carboxy-methylcellulose and poly(vinylpyrrolidone) (PVP) have been prepared by this method [6-10]. However, the need of special facilities for material processing limits its large-scale applicability [11].

The main effect of ionizing radiation in aqueous media is the volumetric generation of hydroxyl radicals ($^{\bullet}OH$) that can abstract H atoms to yield macroradicals [4,5]. Prompted by this consideration, several authors have reported simpler, cheaper methods to produce $^{\bullet}OH$ in water. A plausible route is based on Fenton's reaction chemistry at pH 2.8:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^- \tag{1}$$

PVP nanogels have been prepared by crosslinking linear macromolecules in hexadecyltrimethylammonium bromide reverse micelles [12]. The accumulation of $^{\bullet}OH$ in Fenton systems can be greatly enhanced upon use of UVA radiation and thus, photo-Fenton reaction has been used to generate PVP and polyethyleneimine nanogels [13-15]. Such processes require simpler set-ups than e-beam irradiation but, due the utilization of high concentrations of iron salts (up to 10 mM), the product purification becomes complex since residual concentration of metals must be very low for biomedical applications. On the other hand, the electrochemical advanced oxidation technologies allow

the electrogeneration of different kinds of hydroxyl radicals on site, i.e., adsorbed on the anode surface (Eq. (2)) or free in the solution bulk (Eq. (1)), as demonstrated for the abatement of organic pollutants [16,17].

$$H_2O \rightarrow \ ^{\bullet}OH + H^+ + e^-$$
(2)

This study assesses the performance of three electrochemical approaches characterized by the different nature and reactivity of generated hydroxyl radicals: 1) Electro-oxidation (EO) upon electrogeneration of physisorbed radicals at boron-doped diamond (BDD); 2) EO upon electrogeneration of chemisorbed active oxygen at a dimensionally stable anode (DSA); 3) Electrogeneration of homogeneous $^{\bullet}OH$ by electro-Fenton (EF) process.

In contrast to chemical Fenton methods, much lower concentrations of iron ions (up to two orders of magnitude) are necessary to activate the generation of $^{\bullet}OH$ in EF, thanks to the continuous cathodic reduction of Fe(III) (Eq. (3)) that sustains the catalytic process. Furthermore, the in situ electrogeneration of H_2O_2 (Eq. 4) prevents its dangerous and costly handling [17,18].

$$Fe^{3+} + e^- \rightarrow Fe^{2+}$$
(3)

$$O_2 + 2\ H^+ + 2\ e^- \rightarrow H_2O_2$$
(4)

The aim of our investigation is to provide the proof of concept that at least one of the aforementioned electrochemical routes can be used to change the architecture of linear PVP chains promoting the fabrication of nanogels by intra/intermolecular crosslinking and controlled oxidation/degradation with limited mineralization.

2. Materials and methods

PVP K60 (M_n = 1.6×10^5 g mol^{-1}, 45 wt %, Aldrich) was used as received. A carbon-PTFE air-diffusion electrode (ADE) from E-TEK was used as the cathode in EF to electrogenerate H_2O_2 from pure O_2 gas, whereas Ni was used as cathode in EO. A Ti/IrO$_2$–Ta$_2$O$_5$ (DSA) plate and a BDD thin film supported on Nb, both from ElectroCell AB, were used as anodes. The exposed surface area of all electrodes was 3 cm^2. All potential values are referred to SCE.

For the electrosyntheses of nanogels, fresh PVP aqueous solutions were stirred overnight, filtered with 0.22 μm nylon filters under vacuum and, after deaeration with N_2 for 30 min, stored in opaque, sealed glass bottles. The electrolyses were performed in an undivided glass cell under magnetic stirring, using 50 mL of PVP solution with 50 mM Na_2SO_4 as supporting electrolyte. In EO, N_2 was fed into the solution during the experiments. EF trials were performed at pH 2.8 in the presence of 1.0 mM of FeSO$_4$ as catalyst. An Amel 2053 potentiostat/galvanostat was employed to operate at constant current. Galvanostatic conditions were preferred in order to control the rate of generation of hydroxyl radicals during the whole duration of the electrolyses. Samples were filtered with 1.22 μm cellulose acetate syringe filters. The mineralization of solutions was monitored from their total organic carbon (TOC) decay determined on a Shimadzu LCSH analyser. The hydrodynamic radius (R_h) of polymer chains dispersion was measured after each electrolysis by dynamic light scattering (DLS) using a Brookhaven Instruments BI200-SM goniometer and compared with that of untreated linear PVP (28 ± 9 nm) [19]. DLS data were analyzed according to the method of cumulants [20]. For gel permeation chromatography (GPC) and FTIR analyses, the samples were dialyzed using distilled water for 96 h and employing cellulose acetate membranes (12,000 Da cutoff, Aldrich). GPC analyses of filtered samples were carried out with an Agilent 1100 Series HPLC equipped with two Shodex columns (804 and 806, Phenomenex) in series, using a solution of 3 mM NaN_3 as mobile phase and an RI detector at 35 ºC. FTIR spectra were recorded on a Perkin-Elmer Spectrum 400.

3. Results and discussion

Hydroxyl radicals were first electrogenerated by using BDD, a "non-active" anode producing $^{\bullet}$OH as quasi-free radicals (i.e., physisorbed) [21,22]. BDD presents a low adsorption of species, high corrosion stability, high efficiency in oxidation processes and very efficient generation of quasi-free $^{\bullet}$OH [16]. The latter aspect is particularly relevant, since it has been observed that the weaker the interaction between $^{\bullet}$OH and the anode surface, the higher the O_2-overpotential and the chemical reactivity towards the oxidation of organic molecules. [23] Therefore, in the presence of organics, $^{\bullet}$OH formed from Eq. (2) can cause their partial or total oxidation, which in the case of preformed polymers like PVP could entail crosslinking to yield nanogels of interest.

A first series of experiments was performed at 5 mA cm^{-2}, thus reaching an anode potential of 2.5 – 2.6 V, which is high enough to activate the water oxidation while minimizing the oxygen evolution.

When experiments with different duration (251 and 900 s) and PVP concentration (0.25 or 0.5 wt %) were performed (Table 1, entries 1-3), TOC measurements indicated that no incineration of PVP chains occurred probably due to the low charge passage as compared to that needed for total mineralization. However, none of these trials caused a significant change of PVP R_h, plausibly due to the low amount of radicals generated on the polymer chains. Aiming to stimulate the polymer modification, some experiments were performed under a higher current passage (Table 1, entries 4 and 5). Working at 10 mA cm^{-2} for 1,800 s (entry 4), which corresponds to 54.0 C of charge passed and accounts for 1.5% of that needed for the total mineralization of PVP chains, the R_h still remained unchanged. Furthermore, a small TOC abatement, of the same order of magnitude of that expected on the basis of the charge passed for PVP mineralization was found, which means that $^{\bullet}$OH generated at BDD can abstract H atoms from PVP, although in a useless manner for our purpose since they seem to promote mineralization. This was confirmed when trials were made at 50 mA cm^{-2} for 26,013 s (entry 5), with a charge that corresponds to that for total mineralization. In this case, it was not possible to

obtain a clean DLS signal, which can be explained by the significant degradation of the macromolecules as suggested by the drastic TOC decay.

The EO approach was then tested using an "active" anode like DSA that allows the formation of chemisorbed active oxygen (Eqs. (5) and (6)). In the absence of enough amounts of organic molecules, this oxidant may evolve towards oxygen [16].

$$MO_x + H_2O \rightarrow MO_x(^{\bullet}OH) + H^+ + e^- \tag{5}$$

$$MO_x(^{\bullet}OH) \rightarrow MO_{x+1} + H^+ + e^- \tag{6}$$

Ti/IrO$_2$-Ta$_2$O$_5$ was chosen as optimal DSA since iridium oxide is known to yield chemisorbed active oxygen and, in combination with Ta oxides, presents a good stability [24]. As shown in Table 1 (entries 6-8), no remarkable changes of R_h and TOC content were observed working at low j for different time periods, meaning that the operation conditions were too mild to alter the polymer chains. Conversely, when the electrolyses were performed at 50 mA cm^{-2} with a charge corresponding to that required for total mineralization (entry 9), a significant increase of R_h was achieved, which can be explained by the existence of intermolecular crosslinking promoted under the action of MO$_{x+1}$ that create radical centres on independent PVP chains as they reach the anode. In contrast to BDD (entry 5), TOC content did not undergo a relevant decrease, thus showing that "active" anodes may allow the modification of polymers without causing their mineralization. However, a massive anodic generation of O$_2$ gas was observed because mass transport limitations impede the arrival of enough organic molecules to react with MO$_{x+1}$, which then partly evolves to O$_2$. To minimize the gas evolution, some electrolyses were performed at a very low j (3 mA cm^{-2}) with similar duration. Quite interestingly, analogous results in terms of R_h and TOC content were obtained.

A radically different approach is offered by the EF process, which is usually based on the electrogeneration of H$_2$O$_2$ in aqueous solution via Eq. 4 at massive or gas-diffusion carbonaceous cathodes [17]. Completely free $^{\bullet}$OH in the bulk are formed in this method via Fenton's reaction (1),

whose different nature and space distribution can plausibly lead to a more advantageous modification of the PVP chains. High current densities (33.3 mA cm^{-2}) were always used in EF to ensure the continuous electrogeneration of large amounts of H_2O_2 as well as the cathodic reduction of Fe(III) from Eq. (3) [17]. Experiments were performed at two different concentrations of PVP. A very remarkable decrease of R_h was achieved in both trials (Table 1), which can be accounted for by the intramolecular recombination of free radical centres generated within the polymer chains by $^\bullet$OH in the solution bulk. This agrees with positive findings on PVP crosslinking by non-electrochemical Fenton processes [13-15], now with the additional advantage of needing extremely lower Fe(II) concentrations. Note that, alternatively, scission of macroradical chains could also play its role.

FTIR spectra (Fig. 1) show that both, EO with DSA performed under conditions described in Table 1 (entries 9 or 10 led to analogous IR spectra) and EF treatments (entries 11 and 12) induced an evident higher absorption in the region that can be associated to the stretching of hydroxyl group (3,700-3,000 cm^{-1}), as also reported elsewhere for PVP modification [19,25]. Furthermore, EF process caused the increase of the peak at 1661 cm^{-1} present in the untreated PVP (related to carbonyl stretching of the pyrrolidone ring) and the formation of various new peaks. These included two at 1695 and 1765 cm^{-1}, probably related to the symmetric and asymmetric stretching of a cyclic imide (i.e., succinimide) formed upon hydroxylation of the carbon atom next to the nitrogenated carbon of the ring, and one at 1375 cm^{-1} possibly related to the formation of carboxylate anions or related to imide [19]. Succinimide displays another characteristic band at 820 cm^{-1} due to twisting of -CH$_2$. In experiments with PVP at 0.5 wt % (not shown), the relative absorbance of the peak at 1695 cm^{-1} was lower than that shown in Fig. 1 for 0.25 wt %, which suggests an enhanced radical formation per chain because a lower number of macromolecules compete to react with $^\bullet$OH.

The PVP solutions obtained by EF (Table 1, entries 11-12), which display the most interesting R_h values for biomedical applications, were further analysed by GPC. Narrower elution peaks were

obtained upon electrolyses (Fig. 2), thus indicating a remarkable decrease of the amplitude of the molecular weight distribution. On the other hand, a slightly longer elution time can be observed for the EF trial with PVP at 0.25 wt % (Fig. 2b) compared to that at 0.5 wt %, which suggests the formation of smaller polymer particles, in agreement with the lowest R_h value reported in Table 1 (entry 11). Even if the GPC analyses do not allow ruling out the contribution of chain scission to the process, the reduction of the polydispersity clearly indicates the occurrence of relevant intramolecular and intermolecular crosslinking, essentially for higher and lower molecular weight fractions, respectively.

4. Conclusions

PVP crosslinking is feasible by electrochemical technologies, which are potentially simpler and cheaper than other methods currently available. EO based on quasi-free BDD($^\bullet$OH) appears to be excessively reactive against the polymer chains, causing chain scission with significant mineralization. Oxidation at DSA under selected conditions can lead to higher R_h values, probably due to intermolecular crosslinking, with no significant TOC decay. Conversely, the electrogeneration of totally free $^\bullet$OH in EF seems the most promising technology for preparing nanogels containing quite smaller PVP chains with a low polydispersity of the molecular weight distribution. This work represents the first proof of the concept regarding the modification of the molecular architecture of preformed PVP via electrochemically generated $^\bullet$OH. As occurs in radiolysis, these new alternatives allow performing simultaneously the synthesis and the sterilization of the nanogel, which is highly interesting from an applicative standpoint.

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Figure Captions

Fig.1. In the main plot, FTIR spectra of the untreated PVP at 0.25 wt % (dark, continuous line), PVP treated by EO with DSA (dashed line) under conditions of entry 10 of Table 1, and PVP treated by EF (grey, continuous line) under conditions of entry 11 of Table 1. In the upper left corner, enlargement of the region between 1,800 and 1,300 cm^{-1}, where the arrows indicate new peaks upon treatment by EF.

Fig. 2. GPC analyses of (a) untreated PVP, (b) PVP at 0.25 wt % treated by EF, and (c) PVP at 0.5 wt % treated by EF.

Table 1 Results obtained for the modification of PVP by means of hydroxyl radicals following three different electrochemical approaches

Entry	Process[a]	j (mA cm^{-2}) and E_{cell} (V vs SCE)	Time (s) - Charge passage (C)	[PVP] (wt %)	(Charge passage / Theoretical charge) ratio			R_h[e] (nm)	TOC decay (%)
					Mineralization[b]	1 H per monomeric unit[c]	1 H per chain[d]		
1		5 – 2.6	251 – 3.8	0.25	0.001	0.035	50	32 ± 10	< 1
2	EO with	5 – 2.5	900 – 13.5	0.25	0.004	0.124	179	32 ± 9	< 1
3	BDD	5 – 2.6	251 – 3.8	0.5	0.0005	0.017	25	30 ± 11	< 1
4		10 – 2.7	1,800 – 54.0	0.25	0.015	0.497	716	30 ± 9	2
5		50 – 4.0	26,013 – 3,902	0.25	1.06	35.9	51,756	n.d.	61
6		5 – 1.3	900 – 13.5	0.25	0.004	0.124	179	29 ± 10	< 1
7	EO with	5 – 1.3	3,600 – 54.0	0.25	0.015	0.497	716	32 ± 12	< 1
8	DSA	5 – 1.3	10,800 – 162	0.25	0.044	1.49	2,149	29 ± 9	< 1
9		50 – 2.7	26,013 – 3,902	0.25	1.06	35.9	51,756	55 ± 16	< 1
10		3 – 1.2	26,013 – 234	0.25	0.063	2.15	3,105	54 ± 15	< 1
11	EF	33.3 – n.d.	3,600 –360	0.25	0.097	3.31	4,775	10 ± 5	< 1
12	process	33.3 – n.d.	3,600 –360	0.5	0.049	1.65	2,388	13 ± 5	1-2

[a] All experiments were performed with 50 mM Na$_2$SO$_4$, at room temperature; in EF, 1 mM Fe$_2$SO$_4$ was added to the solution and pH was adjusted to 2.8. [b] Theoretical charge is that needed for the complete mineralization of PVP. [c] Theoretical charge refers to the abstraction of 1 H atom per each monomeric unit. [d] Theoretical charge refers to the abstraction of 1 H atom per each polymer chain. [e] R_h of untreated PVP = 28 ± 9 nm.

Figure 1

Figure 2