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## E-beam crosslinked, biocompatible functional hydrogels incorporating polyaniline nanoparticles

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### ABSTRACT

PANI aqueous nanocolloids in their acid-doped, inherently conductive form were synthesised by means of suitable water soluble polymers used as stabilisers. In particular, poly(vinyl alcohol) (PVA) or chitosan (CT) was used to stabilise PANI nanoparticles, thus preventing PANI precipitation during synthesis and upon storage. Subsequently, e-beam irradiation of the PANI dispersions has been performed with a 12 MeV Linac accelerator. PVA-PANI nanocolloid has been transformed into a PVA-PANI hydrogel nanocomposite by radiation induced crosslinking of PVA. CT-PANI nanoparticles dispersion, in turn, was added to PVA to obtain wall-to-wall gels, as chitosan mainly undergoes chain scission under the chosen irradiation conditions. While the obtainment of uniform PANI particle size distribution was preliminarily ascertained with laser light scattering and TEM microscopy, the typical porous structure of PVA-based freeze dried hydrogels was observed with SEM microscopy for the hydrogel nanocomposites. UV–visible absorption spectroscopy demonstrates that the characteristic, pH-dependent and reversible optical absorption properties of PANI are conferred to the otherwise optically transparent PVA hydrogels. Selected formulations have been also subjected to MTT assays to prove the absence of cytotoxicity.

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### 1. Introduction

Polyaniline (PANI) identifies a rich family of electronically active, mutually converting, conjugated polymers (Kang et al., 1998). Lone electron pairs on nitrogen participate to the conjugation; therefore doping is associated to a chain protonation mechanism. Owing to its amazing electronic properties, coupled to chemical stability and low cost, PANI is the subject of extensive investigation for applications in electronics, opto-electronics and medicine (Guiseppi-Elie, 2010; Pron and Rannou, 2002; Saxena and Malhotra, 2003; Shi and Yeh, 2007; Wallace and Spinks, 2007). What it also makes PANI one of the most studied and yet intriguing conductive polymer is its structure–process–property flexibility by design. The main drawback of polyaniline in its conductive form (protonated emeraldine) is its difficult processability, owing to the low solubility in both aqueous and organic solvents and its infusibility, as well as the intrinsic poor mechanical properties. To overcome these limitations, PANI is often produced in the form of colloidal nanoparticles by recourse to suitable surfactants (Stejskal and Sapurina, 2004) or blended with

other, generally insulating, polymers (Cho et al., 2004) or synthesized in situ onto functionalized substrates (Scaffaro et al., 2011).

For different reasons hydrogels are one other class of materials at the center of research emphasis, finding applications in many fields, from separation to super-absorbents, from tissue engineering to drug delivery (Peppas et al., 2006). In virtue of their 3D, water-rich internal structure, stimuli-responsiveness and biocompatibility, hydrogel molecular structure and properties can be engineered toward the specific application. Purpose of the present work is to develop biocompatible functional polyaniline–hydrogel nanocomposites that combine the electro-optic properties of PANI nanoparticles with process flexibility and highly hydrophilic, 3D structure of the hydrogel matrix for possible application in (bio)sensing or tissue engineering. In particular, PANI nanoparticles in the form of emeraldine salt have been prepared via chemical oxidative dispersion polymerization in an aqueous medium, according to an established procedure (Dispenza et al., 2006a, 2006b), using either polyvinyl alcohol (PVA) or chitosan (CT) as polymeric stabilisers. The hydrogel matrix incorporating polyaniline nanoparticles has been formed in situ by e-beam induced crosslinking of purposely added PVA to the PANI aqueous dispersions containing either PVA–PANI or CT–PANI nanocolloids.

The so produced nanostructured hydrogels have been characterized for their gel fraction and swelling properties at the variance of pH, their morphology and UV–vis absorbance and

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emission properties. Finally, the nanocomposite hydrogels incorporating PANI were subjected to cytotoxicity studies.

## 2. Experimental

PANI aqueous dispersions have been obtained by chemical oxidation of an acid solution of aniline (1 M HCl) using ammonium persulphate as redox initiator and polyvinyl alcohol (PVA, atatic,  $M_v=47,000$ , 88% degree of hydrolysis, Aldrich) or chitosan (CT,  $M_v=50,000$ – $190,000$ , 15–25% degree of acetylation, Aldrich) as steric stabilisers. Details of the synthetic procedure are reported elsewhere (Dispenza et al., 2006a, 2006b). Aqueous dispersions have been subjected to electron beam irradiation in order to induce chemical crosslinking either of the same PVA used to stabilise dispersion (PVA–PANI hydrogel) or purposely added PVA to the CT–PANI nanocolloid (PVA–CT–PANI hydrogel). E-beam irradiation has been performed using a Linac accelerator (ISOF–CNR Bologna, Italy) at an average dose rate of 30 Gy/s and at a controlled temperature of 10 °C. The irradiation dose and the concentration of PVA added to the CT–PANI dispersion prior to irradiation are the result of a preliminary screening carried out by varying both parameters to identify suitable conditions for obtaining macroscopic, wall-to-wall hydrogels. The content of PVA in PVA–PANI hydrogels was maintained the same as in PVA-assisted PANI dispersion polymerisation (4 wt%) while the weight ratio between PVA and PANI was brought to 40. Irradiation dose was varied from 10 to 80 kGy and macrogels were obtained at a dose of 40 kGy and higher. Conversely, chitosan–PANI dispersions, irradiated in the same conditions, did not undergo macroscopic gelation, accordingly to literature (Yoshii et al. 2003). Therefore, PVA was added to the CT–PANI dispersion up to a concentration of 10 wt%, where wall-to-wall gelation with no appreciable syneresis was observed at the highest dose in the investigated range, i.e. 80 kGy.

TEM and SEM microscopy were performed with a JEOL-2100 with an accelerating voltage of 80 kV and a JEOL-FESEM system at an accelerating voltage of 10 kV.

Extraction of soluble portions of the irradiated materials was performed in a Soxhlet apparatus by refluxing water for 8 h. No imparted colouration in the phase containing the extractable was observed. Longer extraction times (up to 24 h) were proved not to increase the weight of extracted fractions. Gel fraction is defined as  $GF=w_g/w_0$ , where  $w_0$  is the total amount of solids in the sample before extraction and  $w_g$  is the weight of the water-insoluble network. The solid content,  $w_0$ , was determined by weighing portions of the hydrogel samples before extraction, after vacuum drying at 60 °C. The reported results are the average of minimum three independent measurements and the experimental error was  $\pm 2\%$ . Swelling studies were carried out on the insoluble portions of the hydrogels as obtained by prolonged immersion (72 h) in excess double-distilled water at  $40 \pm 1$  °C. Swelling Ratios (SR) and Rehydration Ratios (RR) as function of the time were determined. The Swelling Ratio is defined as  $SR=w_s/w_d$ , where  $w_s$  and  $w_d$  are the

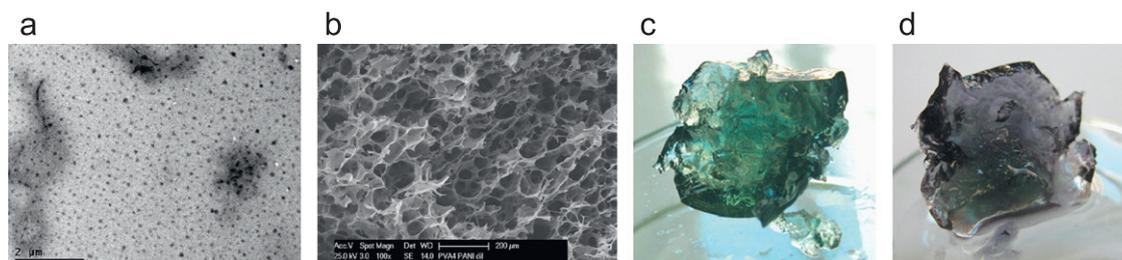
measured weight of the hydrogel in the swollen state and of the freeze-dried residue after the swelling, respectively. Hydrogels were equilibrated either in double distilled water or in an aqueous NaOH solution at pH 9. Rehydration Ratio is defined as the Swelling Ratio, but freeze drying is performed before swelling. Dried samples were put onto pre-weighed cylindrical glass vials with a porous bottom, immersed in a large excess of buffer solutions at  $25 \pm 1$  °C. Phosphate buffers at different pHs (2.5, 6.8 and 9) and same ionic strength (0.2 M) were used. Equilibrium Swelling and Rehydration Ratio (ESR and ERR) values were assumed as the values when SR or RR vs. time curves showed to reach a plateau. Reported SR and RR values are the average of minimum eight samples. The experimental error on the reported SR and RR values is always within  $\pm 2\%$ .

UV–vis absorption spectra were measured by a Jasco V-570 spectrophotometer (scan speed 40 nm/min, integration time 2 s and bandwidth 1 nm) at room temperature. Hydrogel samples were sandwiched between two quartz slides. Fluorescence measurements were carried out with the same sample holder used for absorption spectroscopy using a Jasco FP-6500 spectrofluorimeter equipped with a xenon lamp (150 W). Emission spectra, at the required excitation, were obtained with emission and excitation bandwidth of 3 nm, scan-speed of 100 nm/min and integration time of 1 s, recorded at 0.5 nm intervals.

Viable cell density was assessed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide) assay, accordingly to an established protocol (Dispenza et al., 2011).

## 3. Results and discussion

Polyaniline nanocolloids are synthesised by conventional chemical oxidative polymerisation in the presence of suitable polymeric stabilisers, PVA and chitosan. Reaction conditions are the same as those already reported in a previous paper, using PVP as polymeric stabiliser (Dispenza et al. 2006a, 2006b). Polyaniline in the form of HCl doped emeraldine salt was obtained. Dynamic light scattering (DLS) measurements carried out on the dispersions in the low pH range (1–5) (data not shown) confirm the presence of nanoparticles of about 300 nm diameter, with narrow size distribution. Particles are likely formed by both polyaniline and the suspending polymer. No significant differences have been observed in particle size distribution using PVA or chitosan in this pH range, although stability upon storage at 5 °C of CT–PANI is longer (months). While the particle size distribution is preserved up to pH 7 for PVA, morphology destabilisation occurs at pH 8 and above. CT insolubility in water at  $pH > 6.3$  causes CT–PANI nanoparticles precipitation also in neutral conditions. Fig. 1a shows a TEM micrograph of PANI nanoparticles drop-cast from PVA–PANI dispersion at low pH. PANI nanoparticles are represented by the darker spots, as qualitatively confirmed by EDX analysis showing the presence of the chlorine atoms of the HCl doped emeraldine. The larger aggregates are likely formed upon air-drying of the deposit, as they were not detected in the corresponding dispersion by DLS



**Fig. 1.** TEM micrograph of PVA–PANI nanocolloid (a), SEM micrograph of PVA–PANI hydrogel (b), photograph of PVA–PANI hydrogel at pH 2 (c) and after addition of few drops of concentrated NaOH (d).

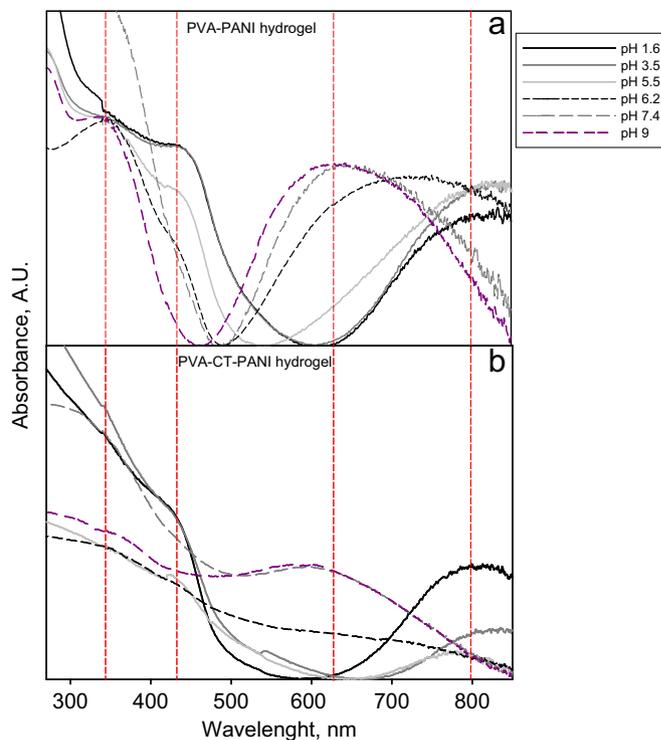
**Table 1**

Gel fractions (GF), Equilibrium Swelling Ratios in water and aqueous sodium hydroxide at pH 9 and Equilibrium Rehydration Ratios in phosphate buffers of different pH for hydrogel nanocomposite and corresponding base hydrogels.

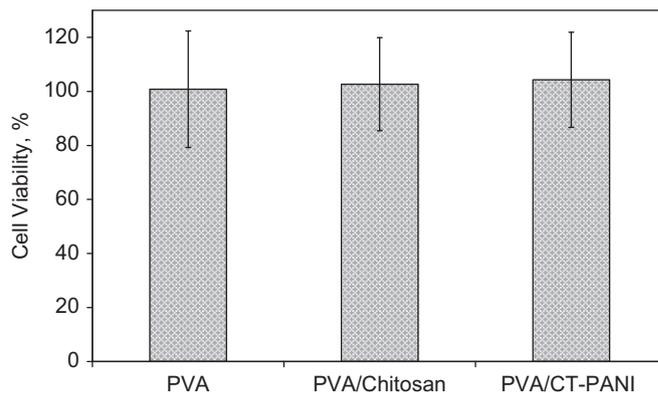
Hydrogel system	GF (%)	ESR		ERR		
		Water	Aq. NaOH pH 9	PBS pH 2.5	PBS pH 6.8	PBS pH 9
PVA (4%)	63.2	17.2	15.3	5.5	9.8	5.1
PANI–PVA (4%)	57.5	19.7	13.1	4.3	8.8	3.8
Chitosan (0.2%)–PVA (10%)	67.5	13.6	6.2	2.5	2.1	2.2
PANI–chitosan (0.2%)–PVA (10%)	58.5	15.6	5.5	2.7	2.6	2.5

measurements. Fig. 1b shows a SEM micrograph of PVA–PANI hydrogel after freeze-drying. PANI nanoparticles, embedded in the PVA matrix, are not visible even at high magnification. At low magnification the typical porous structure of the PVA freeze-dried hydrogel is evident. Fig. 1c–d shows a portion of the PVA–PANI hydrogel, as obtained upon irradiation at 80 kGy (Fig. 1c) and the same after being in contact with few drops of concentrated aqueous sodium hydroxide (Fig. 1d): pH of the hydrogels were measured to be about 2 and 10. At low pH the hydrogel is highly transparent and green coloured, while it transforms into dark blue at alkaline pH. Gel fraction, Equilibrium Swelling Ratios and Rehydration Ratios for the hydrogel nanocomposites and their corresponding base hydrogels at 80 kGy are presented in Table 1. In general, the gel fraction is slightly lower when the network is formed in the presence of polyaniline. The lower GF may be caused by a radical scavenging effect from PANI. Similar effects, yet much more pronounced, were observed for analogous PANI–PVA hydrogels obtained via gamma-crosslinking (Dispenza et al., 2007a). Equilibrium Swelling Ratio values are also slightly higher for the nanocomposite hydrogels than for the base systems and decrease at alkaline pH. With regards to this latter evidence, we can consider that both in distilled water and in PBS at pH 9, polyaniline should be in the unprotonated emeraldine base form, as also confirmed by the colour change from green to blue. The increase in pH does not favour swelling, due to the reduced hydrogen bonding ability of water toward the polar groups of PVA, as well as the reduced charge density of PANI chains. ESR values at pH 9 are significantly smaller for CT containing systems, probably due to the insolubility CT at this pH. Rehydration of freeze-dried hydrogel residues in phosphate buffers at 25 °C does not fully revert the collapsed network structure to fully swollen hydrogels, especially for PVA–CT systems. It can be speculated that irreversible PANI polymer–polymer interactions, which establish in the solid state, cannot be displaced by polymer–water interactions in these conditions.

UV–vis absorption spectra of hydrogels (Fig. 2a–b) present the relevant optical transitions of PANI at the variance of pH. PVA hydrogels do not show any significant absorption in the 390–900 nm region. The HCl doped emeraldine salt in PVA–PANI nanocomposite contributes with a broad band in the visible region at  $\lambda > 700$  nm, owing to PANI polarone structure, with a characteristic absorption at 350 nm for the  $\pi$ – $\pi^*$  transition of the reduced benzenoid repeat unit and with an additional band at 420 nm associated with the doping level of the polymer. Optical transition from green protonated emeraldine to emeraldine base (de-doping) occurs at pH  $> 7$ , as revealed by the disappearance of the absorption at 420 nm and the blue-shift of the band in the visible range. No significant peak shifts are presented by UV–vis spectra of the nanocomposite hydrogel with respect to the parent dispersions (Dispenza et al., 2006b), thus suggesting that e-beam irradiation has preserved the electronic features of PANI nanocolloids. The comparison of the two nanocomposite hydrogels evidences



**Fig. 2.** UV–vis absorption spectra of nanocomposite PANI–PVA hydrogel (a) and PVA–CT–PANI hydrogel (b) at the variance of pH of the swelling medium.



**Fig. 3.** Viability of human dermal fibroblast after 72 h for the base PVA and PVA/chitosan hydrogels and for PVA–CT–PANI hydrogel nanocomposite.

similarities between the two systems at low pH and less distinct bands in the neutral to alkaline range of pH for the PVA–CT–PANI system, due to the partial collapse of the hydrogel network.

Fluorescence spectroscopy reveals a distinct emission band at 325 nm upon excitation at 290 nm from hydrogels containing nanoparticles of PANI in the form of emeraldine salt (the conductive form of PANI), as already observed for the corresponding dispersions and for the PVA–PANI hydrogels produced by gamma-irradiation (Dispenza et al., 2006b, 2007b). Increase in pH to 7 and above quenches the emission signal.

Fig. 3 shows the viability of human dermal fibroblast (HDF) cells in direct contact with the PVA and PVA–CT hydrogels and the PVA–CT–PANI nanocomposite hydrogels.

All the data are compared to the control (cells not in contact with gel), which is arbitrarily taken to be 100%. Tests were carried out after 24, 48 and 72 h of incubation, showing no difference with time. No cytotoxic activity is evidenced by this test.

#### 4. Conclusions

Electron beam has been used to convert PANI-ES nanoparticle dispersions into nanocomposite hydrogels. PANI nanoparticles are bound to the polymer network and do not migrate out upon 72 h immersion in distilled water at 40 °C. The UV–vis absorption and emission properties of PANI nanoparticle dispersions have been preserved. Preliminary biocompatibility studies have demonstrated that the produced materials are not cytotoxic, which encourage a supplement of investigation toward the development of these systems for application in (bio)sensing and smart drug delivery.

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