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Composite gels based on Poly (vinyl alcohol) for biomedical uses

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Abstract

Nowadays, poly (vinyl alcohol) (PVA) hydrogels are being studied for several biomedical applications such as joint replacement, wound dressings and controlled drug-releasing devices, among others. Reinforced PVA hydrogels show good mechanical properties and are a suitable option to replace cartilages. Furthermore, these materials can prevent loss of body fluids, be a barrier against bacteria and also permeable to oxygen, for these all interesting properties, they are used like wound dressings. For drug delivery systems a material that can control the dose and release at the site of action is desirable, this can be accomplished using hydrogels, which are loaded with a drug, and then they can release it when an external stimulus (light, temperature, magnetic field, etc.) takes place. The aim of this work was to obtain composite hydrogels for the previously mentioned biomedical applications. Hydroxyapatite (HA) reinforced PVA gels were prepared for potential uses as cartilage replacement, HA improves the mechanical, tribological and fixing properties of the polymer, reaching values similar to that of the cartilages. For wound dressings, the hydrogel was reinforced with bentonite (clay) in order to increase the dimensional stability and antimicrobial properties. Gels with controlled drug release capability under magnetic stimulation (ferrogels) were also synthesized and characterized here.

Keywords: Poly (vinyl alcohol), composite, hydrogel, biomedical device.

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1. Introduction

In recent years, the interest in using PVA hydrogels has significantly increased, this is related to the high biocompatibility and good gel forming capability of that polymer. Several methods can be used to obtain PVA hydrogels, one of them is the physical method by successive freeze-thaw cycles (FT) which results in a physically crosslinked gel, also called cryogel. The gel structure and stability depend on the numbers of FT [*Riciardi, R. et al 2005 and Hernandez, R. et al 2002*].

However, this material present low chemical resistance and especially poor mechanical properties, limiting their applicability [*Stammen, J.A. et al 2001*]. To overcome these drawbacks, one option is to mix the PVA with other materials [*Park, J.S. el al 2001*].

In tissue replacement area, PVA hydrogel is one of the most promising candidates. PVA hydrogel has good biocompatibility and high elastic modulus, good physicochemical and especially excellent bio-tribological properties [*Kobayashi, M. et al 2005*]. The major problem in such applications is the method of fixation, because this material does not adhere inherently to the tissues. This disadvantage could be overcome by generating a composite hydrogel with a bioactive and biocompatible material such as hydroxyapatite (HA) [*Huang, H-Y et al 1997*].

Hydrogels provide an excellent environment to wound healing; it can absorb exudates generated during the healing process, while protecting the wound from secondary infection [Gwon*, H-J. et al 2009*]. Moreover, nanocomposites based on PVA and clay have shown significant improvements in mechanical and other properties (eg, increased mechanical strength and heat resistance and decreased gas permeability and flammability [*Sinha Ray, S. et al 2003*]) compared to the neat polymer (or matrix) or macro and micro composites, so that these nanocomposites appear to be an adequate material for bandages.

Ferrogels are a new class of polymeric materials whose properties can be controlled by applying a magnetic field. The magneto-elastic properties of these gels give potentiality for applications as biomembranes, biomedical materials, biosensors, artificial muscles and drug delivery devices [*Paradossi, G. et al 2003 and Matejka, L. et al 2004*].

The objective of this work was to obtain composite hydrogels for uses as replacement joints, wound dressings and drug-releasing systems. Therefore, in order to obtain gels with characteristics similar to articular cartilage, the PVA was reinforced with HA (PVA/HA gels) attempting to improve its mechanical, tribological and fixing (for the osteo-conductive character of the HA) properties. In the case of hydrogels for use as wound dressings, bentonite was added (PVA/Bentonite gels) for improving the dimensional stability, water vapor permeability and the antimicrobial properties of the material. Furthermore to obtain gels with ability to modify its dimensional configuration with a magnetic stimulation (ferrogels), magnetic iron oxides was "in situ" synthesized for use as drug-releasing systems.

2. Materials and Methods.

2.1. Materials.

PVA with a hydrolysis degree higher than 98-99% and a molecular average weight of 89000-98000 g/mol was purchased from Aldrich. Iron (II) sulfate heptahydrated and Iron (III) chloride hexahydrated were obtained from Cicarelli laboratory (Argentina), glutaraldehyde was from QRS analytical (Argentine) hydrochloric acid and methanol were from Biopack, (Argentina), acetic acid buffer, sulphuric acid, and ammonium hydroxide were from Anedra laboratory (Argentina). The bentonite was supplied by Minarmco S.A. (Neuquén, Argentina). For the synthesis of PVA/HA the following reagents were used: Calcium hydroxide, $Ca(OH)_2$ (98.5% Biopack); phosphoric acid, H_3PO_4 (density: 1.71g/ml, Merck) and distilled water.

2.2. Preparation of composites gels.

2.2.1. PVA/HA gels.

PVA/HA hydrogels were synthesized from aqueous solutions of 15 wt. % PVA. First, the necessary amounts of PVA and $Ca(OH)_2$ were dissolved in distilled water and stirred at 85 °C. After an hour of stirring, the required amount of H_3PO_4 was added under continuing agitation for three hours. For the studied theoretical HA concentration (3 wt. %) a stoichiometric ratio of Ca/P = 1.67, which satisfies the synthesis reaction of HA, was employed. Then the mixture was allowed to reach room temperature (RT) and placed in an ultrasonic bath for 30 minutes to remove trapped air. The mixture was poured into plastic moulds and three cycles of FT (freezing at -18 °C and thawing at 25 °C) were performed (12 h per stage).

2.2.2. PVA/Bentonite gels.

Aqueous solutions of 15 wt. % PVA were prepared by dissolving the polymer in distilled water at 85 °C and slowly stirring (with magnetic stirrer). After an hour of mixing, 3 wt. % of bentonite was added, the agitation continued for three hours and the polymer was entirely dissolved. After that, the solutions were allowed to reach RT and then placed in an ultrasonic bath for 30 minutes to remove all bubbles. Then, the PVA-based solutions were cast onto anti-adherent containers and the same FT procedure previously described was followed.

2.2.3. PVA/iron oxides gels.

A solution with 10 wt. % of PVA and 6 wt. % of $Cl_3Fe.6H_2O$ - $SO_4Fe.7H_2O$ (2:1) (in presence of HCl to avoid oxidation of Fe (II)) was prepared and was stirred at 85 ºC during 4 h. After cooling to room RT, NH_3 was added to the clear solution to produce the basic co-precipitation (pH = 10) of magnetite (Fe_3O_4) nanoparticles (NPs). The reaction is shown in the next scheme equation (1).

$$Fe^{+2} + 2\,Fe^{+3} + 8\,OH^{-} \rightarrow Fe_3O_4 + 4\,H_2O \qquad (1)$$

Crosslinking was carried out on flat moulds using aliquots of 10 ml of the initial solution and the FT procedure was carried out as it was described before.

2.3. Methods.

2.3.1. Scanning Electron Microscopy (SEM).

In order to analyze the composites morphology SEM micrographs were obtained in a JEOL JSM-6460 LV instrument. The samples were cryo-fractured in liquid air and then gold-coated.

2.3.2. Field Emission Scanning Electron Microscopy (FESEM).

Ferrogel morphologies were observed by FESEM in a Zeiss ULTRA plus instrument. The samples were lyophilized and fractured in liquid air before testing.

2.3.3. Differential Scanning Calorimetry (DSC) studies.

Measurements were carried out in a Shimadzu DSC-50. Samples were scanned from room temperature to 260 ºC at a heating rate of 10 ºC/min, under nitrogen atmosphere. Before DSC analysis, gels samples were dried for 48 h at 37 ºC. The melting temperature (T_m) was determined from the obtained curve. The degree of crystallinity ($X_{cr}\%$) was calculated from the following equation:

$$X_{cr}\% = \frac{\Delta H}{\Delta H^0 \times W_{PVA}} \times 100 \qquad (2)$$

where ΔH was determined by integrating the area under the melting peak over the range 190-240 °C and ΔH^0 was the heat (138.6 J/g) required for melting a 100% crystalline PVA sample [*Peppas, N. et al 1976*] and

W_{PVA} is the mass fraction of the matrix.

2.3.4. X-Ray Diffraction (XRD).

XRD analysis was carried out using an Analytical Expert Instrument ($\lambda_{CuK\infty}$=1.54Å) from 2θ = 3 º to 60 º at a rate of 2 º/min.

2.3.5. Thermogravimetric Analysis (TGA).

Thermogravimetric studies were carried out in a Shimadzu TGA-DTGA 50. All dried samples (37 ºC, 48h) were run from RT to 900 °C at 10 °C/min under air atmosphere. The bound water content ($H_2O\%$), the degradation temperature (T_P) and the reinforcement content (*I%*) were obtained from such TGA curves.

2.3.6. Gel fraction determinations.

To perform gel fraction (*GF%*) measurements, a slice of each sample was placed in a oven before and after rinsing at 37 ºC until no change in its mass was observed. After that, each sample was immersed into distilled water at room temperature for 4 days to rinse away un-reacted species. Subsequently, the immersed sample was removed from distilled water and dried at 37 ºC until constant weight was reached. Therefore the gel fraction can be calculated as follows:

$$GF\% = \frac{W_f - W_F}{W_i - W_F} \times 100 \qquad (3)$$

where W_i and W_f are the weights of the dried hydrogels before and after immersion respectively, and W_F is the weight of the used filler.

2.3.7. Swelling Studies.

Swelling determinations were carried out in saline solution (0.90 % w/v of NaCl in water) in a thermostatic water bath at 37 ºC. All samples were dried before immersion at 37 ºC for 48 h. The swelling percentage (M_t *%*) was determined by the following equation:

$$M_t\% = \frac{M_f - M_i}{M_i} \times 100 \qquad (4)$$

where M_i and M_f are the weights of the sample before and after immersion respectively.

2.3.8. Compression test.

Compression tests were conducted on the hydrogels of PVA and PVA/HA in a universal testing machine Instron 4467 at room temperature and a crosshead speed of 5 mm /min. The samples were swollen in saline solution, at 37 °C for 72 hours approximately (until equilibrium weight was reached) before tests. Young's modulus (*E*), the tensile strength (σ_m) and the final strain ($\varepsilon_r\%$) to rupture were obtained.

2.3.9. Tensile test.

Tensile tests were performed in hydrated (with equilibrium water content, M_∞) and dehydrated films (dried until constant weight, 37 ºC). Tests were performed in a universal testing machine Instron 4467 at room temperature and a crosshead speed of 50 mm/min.

2.3.10. Friction measurements.

Friction tests were conducted, based on ASTM D1984-01 standard, on PVA and PVA/HA hydrogels in a universal testing machine Instron 4467 at room temperature and a crosshead speed of 18 mm/min.

2.3.11. Antimicrobial assays.

Antibacterial activity tests were made using the agar diffusion method according to Ponce et al [*Ponce, A.G. et al 2003]*. The zone of inhibition assay on solid media was used for determination of the antibacterial effects of films against a Gram negative and positive bacterialike *Escherichia coli O157:H7 and Listeria monocytogenes* innocua respectively. Gram is the most common and well-known classification of bacterias, they can be Gram positive or negative depending on the composition of the bacteria cellular membrane. Hydrogels were cut into a disc form of 5 mm diameter and then placed on Mueller Hinton (Merck, Darmstadt, Germany) agar plates, which had been previously seeded with 0.1 ml of inoculums containing approximately 10^5 - 10^6 CFU(colony-forming unit)/ml of tested bacteria. The plates were then incubated at 37 ^{o}C for 24 h. After that, the plates were examined for 'zone of inhibition' on the film discs. The diameters of inhibitory zones surrounding hydrogels discs were measured. The antibacterial effect of hydrogels was classified by the diameter of the inhibition halos as: not sensitive, for diameters less than 17 mm; sensitive, for diameters of 18-23 mm; very sensitive, for diameters of 24-28 mm and extremely sensitive, for diameters larger than 29 mm. Each assay was performed by triplicate on two separate experimental runs.

2.3.12. Water vapor transmission rate (WVTR)

This procedure was performed following the European pharmacopeia standard [*Kokabi, M. et al 2007*].For these measurements, dried hydrogels (maintained in a humidity chamber at 60% for 10 days, until constant weight) samples with a diameter of 28 ± 2 mm and a thickness of 0.16 ± 0.04 mm were cut and then put as a cap with adhesive on the mouth of a flask with a diameter of about 26 mm containing 20 ml of distilled water. The flask was then placed in a constant temperature-humidity homemade chamber for 72 h (37ºC at 75% humidity). The homemade chamber was built as it is described in ASTM D5032-97 (2003) standard. The mass loss of the system was considered as an index of WVTR. The WVTR of each sample was calculated by using the following equation:

$$WVTR\left(\frac{g}{m^2 \times h}\right) = \frac{M_0 - M_1}{72 \times A} \times 10 \qquad (5)$$

where A is the area of flask mouth (mm^2), M_0 and M_1 are the mass of the system (flask and hydrogel cap) before and after placing in the chamber, respectively.

2.3.13. Magnetic Properties.

They were investigated using a SQUID (superconducting quantum interference device). Magnetization of ferrogel samples was measured at room temperature (25 ºC) as a function of the applied magnetic field between -20 kOe to 20 kOe.

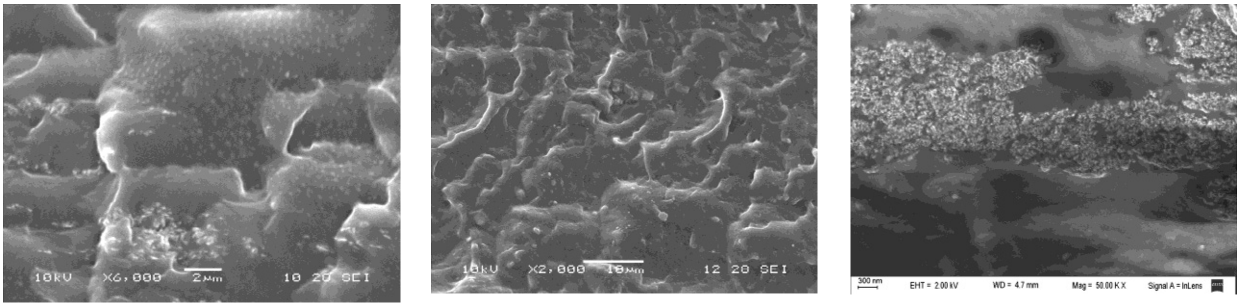
3. Results and Discussions.

In the following Table 1 are listed the results obtained in the thermal and morphological characterization of the composites.

Table 1. Thermal and morphological characterization of composites.

Gel	T_m(°C)	X_{cr}(%)	I(%)	H_2O(%)	GF(%)	M_∞(%)
PVA	227.3	44.6	-	7.3	72.2 ± 3.1	146.6 ± 1.5
PVA/HA	229.7	38.3	3.6	6.9	76.9 ± 3.4	141.8 ± 9.7
PVA/Bentonite	227.3	45.4	2.8	8.0	93.9 ± 0.7	164.3 ± 2.2
Ferrogel	215.0	26.2	6.7	6.6	84.2 ± 0.6	107.5 ± 3.7

Figures 1 a, b and c show the micrographs for the composites where it can be observed the HA particles, bentonite and magnetite within the matrix of PVA.



a) b) c)

Figure 1. Micrograph of composites: a) PVA/HA by SEM with a magnification of 6000x and b) PVA/Bentonite by SEM with a magnification of 2000x and c) Ferrogel by FESEM with a magnification of 50000x.

Electronic micrographs (Figure 1) and also XRD spectra (not shown), indicated that it was possible to synthesize by "in situ" technique, both nanoparticles: magnetite (with diameter below 50 nm) and HA (with diameter 140 nm) and further that all used reinforcements were very well dispersed and distributed in the matrix.

Moreover, the specific properties were analyzed depending on the potential uses of each material and the obtained parameters are shown in Table 2.

Table 2. Composite description and their specific properties.

Gel	Potential use	Specific assay	Result
PVA/HA	Joint replacement	Compression	E=148.6 ± 13,1 MPa
			σ_m >29.2 MPa
			ε_r >43.0%
		Tensile	E= 275 ± 23 kPa
			σ_m= 721 ± 25 kPa
			ε_r= 468.8±48.4 %
		Friction	μ=0.067±0.049
PVA/Bentonite	Wound dressing	Permeability	WVTR =7.71 ± 0.52 g/(h.m^2)
		Tensile	E=53 ± 18 kPa
			σ_m=441± 167 kPa
			ε_r=899.7 ± 316.8 %
		Antimicrobial properties	Extremely sensitive to *Listeria* and sensitive to *E. coli*
Ferrogel	Drug release system	XRD	Magnetite peaks are found.
		Magnetic measurement	Superparamagnetic behavior without coercitivy. Saturation magnetization = 3,6 emu

In the case of the hydrogels with HA, the results indicate that the inclusion of phosphates in the matrix produces a decrease on the degree of crystallinity and water uptake (Table 1). The frictional properties of the material depend on the mechanical properties and water content of the hydrogel. Thus, PVA/HA could be used for potential articular cartilage replacement.

Regarding biomaterials for wound healing, it is known that the ideal rate of evaporation is the one that allows the wound to breathe, but also provides an enabling environment for the migration of the cells responsible for healing, absorbing the generated exudates. The optimal *WTVR* for bandage materials should be between the values for the normal skin (8.5 g/m^2 per hour) and diseased skin (which can reach up to 11.6 g/m^2 per hour) [*Mi, F-L et al 2001*]. Table 2 shows that the results obtained for PVA/bentonite hydrogels are in this range: in addition it is also possible to observe that the *GF* and M_∞ remain high, indicating the potentiality of developed materials for use in healing wounds.

The above table displays that the obtained ferrogel exhibits superparamagnetic behavior at room temperature with very low values of coercivity (10 Oe) which is attributed to small fractions of particles but large enough to produce blocking at room temperature.

4. Conclusions.

The synthesized compounds hydrogels would be able to act as biomedical materials in the applications proposed in this paper. The reinforcements substantially improve the specific properties required for each application with respect to the neat PVA matrix.

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References

Gwon, H-J, Lim, Y-M, An, S-J, Youn, M-H, Han, S-H, Chang, H-N, Nho. Y-C., 2009. Characterization of PVA/glycerin hydrogels made by γ-irradiation for advanced wound dressings. Korean J. Chem. Eng. 26, p. 1686-1688.

Hernandez, R., Lopez, D., Mijangos, C., Guenet, J.M., 2002. A reappraisal of the 'thermoreversible' gelation of aqueous poly(vinyl alcohol) solutions through freezing–thawing cycles. Polymer 43, p. 5661–5663.

Huang, H.Y., Liu, Z.H., Tao, F., 1997. In vivo evaluation of porous hydroxylapatite ceramic as cervical vertebra substitute. Clinical Neurol. Neurosurg 99, p. 20–21.

Kobayashi, M., Chang, Y.S., Oka, M., 2005. A two year in vivo study of polyvinyl alcohol-hydrogel (PVA-H) artificial meniscus. Biomaterials 26, p. 3243–3248.

Kokabi, M., Sirousazar, M., Hassan, Z., 2007. PVA–clay nanocomposite hydrogels for wound dressing. European Polymer Journal 43, p.773–781.

Matejka, L., Dukh, O., Hlavata, D., Meissner, B., Brus, J., 2004. Cyclization and Self-Organization in Polymerization of Trialkoxysilanes. Macromolecules. 36, p. 7977-7983.

Mi, F-L, Shyu, S-S, Wu, Y-B, Lee, S-T, Shyong, J-Y, Huang, R-N., 2001. Fabrication and characterization of a sponge-like asymmetric chitosan membrane as a wound dressing. Biomaterials 22, p. 165-173.

Paradossi, G., Cavalieri, F., Chiessi, E., Spagnoli, C., Cowman, M.K., 2003. Poly(vinyl alcohol) as versatile biomaterial for potential biomedical applications. J. Mater. Sci. Mater. Med. 14, p.687-699.

Park, J.S., Park, J.W., Ruckenstein, E., 2001.Thermal and dynamic mechanical analysis of PVA/MC blend hydrogels. Polymer 42, p. 4271-4280.

Peppas, N.A., Merrill, E.W., 1976. Differential scanning calorimetry of crystalline PVA hydrogels. Journal of Applied Polymer Science 20, p. 1457-1465.

Ponce, A.G., Fritz, R., Del Valle, C.E. and Roura, S.I., 2003. Antimicrobial activity of essential oils on native microbial population of organic Swiss chard. Lebensmittel-Wissenschaft und Technology 36, p. 679–684.

Ricciardi, R., Mangiapia, G., Lo Celso, F., Paduano, L., Triolo, R., Auriemma, F., De Rosa, C., Lauprêtre, F., 2005. Structural Organization of Poly(vinyl alcohol) Hydrogels Obtained by Freezing and Thawing Techniques: A SANS Study. Chemistry of Materials 17, p. 1183-1189.

Stammen, J.A., Williams, S., Ku, D.N., Guldberg, R.E., 2001.Mechanical properties of a novel PVA hydrogel in shear and unconfined compression. Biomaterials 22, p. 799–806.

Sinha Ray, S., Okamoto, M., 2003.Polymer/layered silicate nanocomposites: a review from preparation to processing. Progress in Polymer Science 28, p.1539-1641.