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A new biodegradable and biocompatible hydrogel with polyaminoacid structure

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Abstract

The preparation and physicochemical and biological characterization of a novel polyaminoacid hydrogel have been reported. The α,β-poly(*N*-2-hydroxyethyl)-DL-aspartamide (PHEA) has been used as a starting polymer for a derivatization reaction with methacrylic anhydride (MA) to give rise to the methacrylate derivative named PHM. Photocrosslinking of PHM has been performed in aqueous solution at 313 nm and in the absence of toxic initiators. PHM-based hydrogel has been characterized by scanning electron microscopy, X-ray diffractometry, swelling measurements in aqueous media; the degradation of PHM-based hydrogel has been evaluated as a function of time in the absence or in the presence of esterase. Besides, the biocompatibility of this hydrogel and of its degradation products has been evaluated by performing *in vitro* assays on human chronic myelogenous leukaemia cells (K-562), chosen as a model cell line. Finally, ATR-FTIR measurements have showed that interaction between PHM-based hydrogel and each of four plasma proteins (albumin, γ-globulin, transferrin and fibrinogen) does not cause change in protein conformation thus supporting its potential use as a material to prepare parenteral drug delivery systems.

Keywords: Polyaspartamide; Photocrosslinking; Biodegradable hydrogels; ATR-FTIR; Plasma proteins

1. Introduction

Hydrogels, i.e. polymeric three-dimensional networks able to swell in the presence of an aqueous medium, are extensively employed in biomedical and pharmaceutical field as soft contact lenses, wound dressing, drug delivery systems, biosensors and implantable devices in tissue engineering (Blanco et al., 2000; Bryant and Anseth, 2000; Maris et al., 2001; Mellot et al., 2001; Soppimath et al., 2001; Ju et al., 2001; Gan et al., 2001; Lloyd et al., 2001; Razzak et al., 2001; Quintanar-Guerrero et al., 2001).

Most commonly polymers employed to prepare hydrogels include poly(hydroxyethylmethacrylate) (PHEMA); (polymethylmethacrylate) (PMMA); polyacrylic acid (PAA); poly(diethylaminoethylmethacrylate) (PDEAEMA). However, these synthetic polymers are not biodegradable by hydrolytic or enzymatic mechanisms and this is a limitation for their application as implantable devices or biosensors. On the contrary, the use of biodegradable polymers, such as polyaminoacids, proteins and polysaccharides gives the opportunity of preparing more versatile hydrogels (Brondsted et al., 1998; Park et al., 1998; Markland et al., 1999). In particular, polyaminoacids are an interesting class of materials because of their protein-like nature and the possibility of preparation via chemical or biosynthetic routes with the proper molecular weight and a narrow molecular weight distribution. Besides, it is possible to synthesize polyaminoacids containing functional groups suitable for: (i) chemical functionalization to prepare various derivatives (polymeric surfactants, polycations, polymeric ligands); (ii) conjugation with active molecules, for example, drugs to prepare macromolecular prodrugs; (iii) crosslinking to prepare hydrogels.

However, in despite of their several advantages, the preparation of polyaminoacids on large scale can cause complex technical and economic problems. For this reason, few sci-

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entific papers report the preparation of hydrogels based on polyaminoacids such as poly(aspartic acid), poly(L-lysine), poly(L-glutamic acid), poly(hydroxyethyl-L-glutamate) and poly(L-ornithine) (Dickinson et al., 1981; Yamamoto and Hirata, 1995; Kunioka and Choi, 1995; Gonzales et al., 1996; Tomida et al., 1997; Ohkawa et al., 1998).

Fortunately, the thermal approach for synthesizing polyaminoacids, by dry heating of starting monomers, is a promising approach to overcome these drawbacks. On this point, the synthesis of analogues derivatives of poly(aspartic acid) is of particular interest. Among them, the α,β-poly(*N*-2-hydroxyethyl)-DL-aspartamide (PHEA) is a synthetic polymer obtained by a simple aminolysis with ethanolamine of a polysuccinimide (PSI), in its turn easily synthesized by thermal polycondensation of D,L-aspartic acid.

PHEA is a water soluble and biocompatible polymer already proposed as a plasma expander, carrier for drugs and starting material to prepare polycations for gene delivery and chemical hydrogels (Giammona et al., 1997, 1999a,b, 2001; Cavallaro et al., 2001, 2004; Pitarresi et al., 2004; Licciardi et al., 2006).

In this work, we have prepared and characterized a novel chemical hydrogel obtained by UV irradiation of a PHEA derivative, named PHM, containing in the side chains photocrosslinkable groups such as methacrylate functions.

The hydrogel has been extensively characterized from the physicochemical and biological point of view including swelling ability, susceptibility of chemical and enzymatic hydrolysis and cell compatibility. Finally, in order to obtain some preliminary information on the possible use of this material for parenteral administration, the interaction between this hydrogel and some plasma proteins has been evaluated by using the attenuated total reflection infrared spectroscopy (ATR-FTIR).

2. Materials and methods

2.1. Materials

All reagents were of analytical grade, unless otherwise stated. D,L-Aspartic acid, ethanolamine, anhydrous *N*,*N*-dimethylacetamide (DMA), methacrylic anhydride (MA) and triethylamine (TEA) were from Fluka (Italy). Albumin from human serum (99%), γ-globulin from human blood (99%), human transferrin (≥98%) and fibrinogen from human plasma (50%) are from Sigma (Italy).

α,β-Poly(*N*-2-hydroxyethyl)-DL-aspartamide (PHEA) was prepared by reaction of a polysuccinimide (PSI), obtained by thermal polycondensation of D,L-aspartic acid, with ethanolamine in DMF solution, purified and characterized according to a previously reported procedure (Giammona et al., 1987). The batch of PHEA used in the present study had a weight–average molecular weight of 56.9 kDa (M_w/M_n 1.79), determined by size exclusion chromatography (SEC) analysis.

2.1.1. Cell culture

Human chronic myelogenous leukaemia cells (K-562) were grown in RPMI-1640 medium (Sigma, Milan, Italy) supplemented with 10% fetal calf serum (Sigma, Milan, Italy), 1% (v/v) penicillin–streptomycin solution and 1% (v/v) amphoterycin B solution, incubated at 37 °C in a humidified atmosphere containing 5% CO_2.

2.2. Apparatus

FT-IR spectra were recorded as pellets in KBr in the range 4000–400 cm^{-1} using a Perkin-Elmer 1720 Fourier Transform Spectrophotometer with a resolution of 1 cm^{-1}; each spectrum was recorded after 100 scans.

UV irradiation was performed by using a Rayonet reactor equipped with a Rayonet Carousel motor assembly and 16 mercury lamps of 8 W at medium pressure with an emission at 313 nm.

Centrifugations were performed with an International Equipment Company Centra MP4R equipped with a 854 rotor and temperature control.

Scanning electron microscopy (SEM) was performed with a Leo stereoscan 420; the sample surface was made conductive by the deposition of a layer of gold in a vacuum chamber.

X-ray diffraction analysis was performed using a diffractometer Philips PW 1729 X-ray generator diffractometer. The experimental parameters were set as follows: Cu Kα radiation, tube setting 40 kV, 20 mA; angular speed 2° (2θ/min); range recorded 10–60° (2θ/min); time constant 1 s, chart speed 2 cm/min.

2.3. PHM synthesis

The derivatization reaction of α,β-poly(*N*-2-hydroxyethyl)-DL-aspartamide (PHEA) with methacrylic anhydride (MA) to obtain PHM copolymer, was carried out in organic phase (anhydrous DMA), using TEA as a catalyst, according to a previously reported procedure (Mandracchia et al., 2004). In particular, 2 g of PHEA were dissolved in 40 ml of anhydrous *N*,*N*-dimethylacetamide (DMA) and then suitable amounts of triethylamine (TEA) and methacrylic anhydride were added, according to $X = 0.5$ and $Y = 0.5$, being

$$X = \frac{\text{moles of methacrylic anhydride}}{\text{moles of PHEA repeating unit}},$$

$$Y = \frac{\text{moles of TEA}}{\text{moles of methacrylic anhydride}}$$

The reaction was kept at 40 °C under continuous stirring for 48 h. After this time, the reaction mixture was precipitated with 2-propanol and centrifuged for 10 min, at 12,000 × *g* and 4 °C. The product was recovered by filtration, washed four times with 2-propanol and four times with acetone, then dried under vacuum.

The obtained PHM copolymer was dissolved in twice-distilled water and subjected to extensive dialysis by using Visking Dialysis Tubing (18/32 in.) with a molecular weight cut-off of 12,000–14,000. After dialysis, the solution was dried by freeze-drying. PHM was obtained with a yield of 97–99% (w/w), based on the starting PHEA and characterized by FT-IR

and ^{1}H NMR analyses. Spectral data were in agreement with the attributed structure (Mandracchia et al., 2004).

The degree of derivatization (DD), determined by ^{1}H NMR analysis, resulted to be 30 ± 1 mol%.

2.4. Preparation of PHM-based hydrogel

An aqueous solution of PHM (60 mg/ml) was placed in a Pyrex tube equipped with an internal Pyrex piston in order to have a sample of about 2 mm in thickness, then irradiated with a UV source at 313 nm under argon for 3.5 h.

After irradiation, the obtained hydrogel of PHM was purified by several washing with twice-distilled water, centrifuging, from time to time, at 12,000 × *g* and 4 °C for 15 min, then recovered after freeze-drying.

The experiment was performed in triplicate and the mean yield % (based on the starting amount of PHM) resulted to be 89% (w/w).

PHM-based hydrogel was characterized by FT-IR spectrophotometry. In particular, FT-IR spectrum (KBr) showed a broad band centred at 3300 cm^{-1} (ν_{as} OH + ν_{as} NH), bands at 1730 cm^{-1} (ν_{as} COO), 1656 cm^{-1} (Amide I), 1543 cm^{-1} (Amide II) and 1170 cm^{-1} (ν_s COO).

2.4.1. Swelling studies

Swelling ability of PHM-based hydrogel was determined in twice-distilled water and phosphate buffer (NaCl, Na_2HPO_4, KH_2PO_4) at pH 7.4 (simulated physiological fluid).

In particular, aliquots exactly weighed of the hydrogel were placed in tared 5 ml sintered glass filters (Ø 10 mm; porosity, G3) and left to swell at 37.0 ± 0.1 °C by immersing the filters plus supports in beakers containing liquid medium. After suitable time intervals the excess of liquid was removed by percolation at atmospheric pressure. The filter was placed in a properly sized centrifuge test tube then centrifuged at 700 × *g* for 5 min and weighed. The filter tare was determined after centrifugation with water alone. The weight swelling ratio (*q*) was calculated as follows:

$$q = \frac{W_s}{W_d}$$

where W_s and W_d are the weights of the swollen and dry sample, respectively.

Each experiment was carried out in triplicate and the results were in agreement within ±2% standard error.

2.4.2. Chemical and enzymatic hydrolysis studies

Aliquots of the PHM-based hydrogel (30 mg) have been dispersed in phosphate buffer (NaCl, Na_2HPO_4, KH_2PO_4) at pH 7.4 in the absence or in the presence of esterase (final enzyme concentration 0.5 mg/ml) at 37.0 ± 0.1 °C under continuous stirring (100 rpm) for 1, 3, 5, 10 and 20 days. For the experiments performed in the presence of esterase, a new dose of enzyme was added after each 24 h. Enzyme solution was prepared immediately before the experiment. After each chosen time, the samples were centrifuged at 12,000 × *g* at 4 °C for 15 min and the supernatant was separated. For each sample, the remaining hydrogel was washed several times with twice-distilled water under continuous stirring for 1 h at 37 °C to extract soluble polymer degradation products, electrolytes and enzyme entrapped in the network. Finally, the hydrogel was recovered by freeze-drying, weighed and characterized by swelling measurements in twice-distilled water.

Each experiment was performed in triplicate and the results were in agreement within ±3% standard error.

2.5. Cytotoxicity assay

2.5.1. Indirect method

Detection of cytotoxic effects of PHM-based hydrogel was performed indirectly using the growth medium conditioned by the biomaterial.

At the beginning, the biomaterial was incubated in RPMI-1640 medium without fetal calf serum (FCS) at 37 °C for 120 h into a 25 cm^2 flask. After incubation, the medium was centrifuged at 12,000 × *g*, 4 °C for 30 min and later sterilely filtrated in order to remove hydrogel.

K-562 cells were suspended at a density of 1 × 10^5 cells/ml in RPMI-1640 conditioned medium and supplemented with 10% FCS, transferred to 24-well plate (1 ml per well), and incubated at 37 °C for 48 h.

The assay was performed using a growth medium both 100% conditioned and 8% (dilution 1:12 with RPMI-1640) conditioned. Viability of cells was assessed by the Trypan blue exclusion assay.

After incubation, cells were diluted 1:1 with Trypan blue solution (0.4%, w/v) and transferred into the Burker chamber. In this experiment, the cells with damaged cytoplasm membranes were coloured and determined in sight field of microscope. Viability was expressed as a percentage of the uncoloured cells.

In control experiments, cells were incubated in RPMI-1640 not conditioned by the hydrogel.

Each experiment was performed in triplicate and the results were in agreement within ±5% standard error.

2.5.2. Direct method

PHM-based hydrogel was tested *in vitro* for its activity against K-562 cells using a "direct contact" assay. The cells were suspended at a density of 1 × 10^5 cells/ml in RPMI-1640 medium and directly incubated with hydrogel sample kept on the well bottom of a 24-well plate.

Viable cells number was calculated in a hematocytometer by dye exclusion with Trypan blue after incubation for 48 h. The decrease in cell viability caused by hydrogel was estimated in terms of percentage of cell viability. In control experiments, cells were incubated in RPMI-1640 medium in the absence of the hydrogel.

Each experiment was performed in triplicate and the results were in agreement within ±5% standard error.

2.6. ATR-FTIR spectroscopy

To evaluate the interaction between the PHM-based hydrogel and some plasma proteins, a FTIR spectrometer (Vector 22

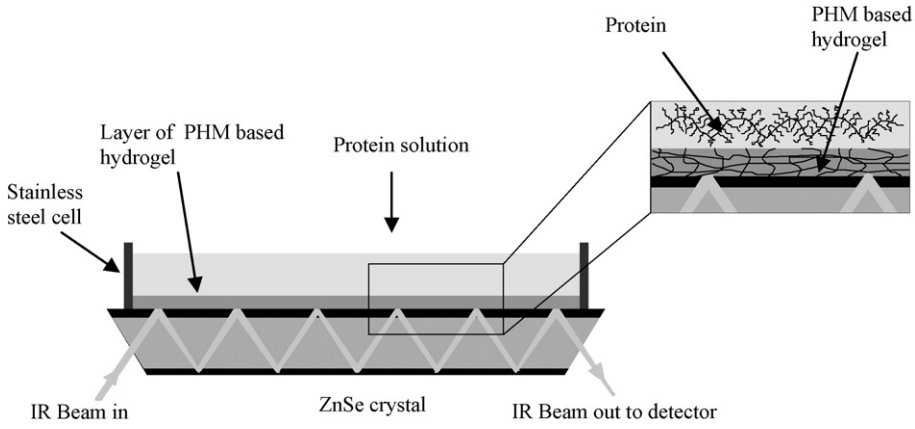


Fig. 1. Schematic representation of the ATR-FTIR experimental arrangement.

Bruker) with a horizontal ATR accessory (Specac) and equipped with a cover to prevent solvent evaporation, was used in the configuration shown in Fig. 1.

This arrangement allows to IR beam to enter the layer of gel to a small fixed depth and to be specifically attenuated according to the molecules that are in this region. The ATR crystal was ZnSe with an angle of incidence of 45°, 50 mm long, 10 mm wide and 2 mm thick. The cell and ZnSe crystal were sealed together by using a petroleum gel and the joints monitored for leaks. The measurements were performed under nitrogen in order to avoid the interference of water vapour and carbon dioxide. A phosphate buffer solution at pH 7.4 in contact with ZnSe crystal was always used as a background for all the experiments. A sample of swollen PHM-based hydrogel (containing 20%, w/v in polymer and 80%, w/v in water) was placed on the crystal in order to have a layer whose thickness was measured at multiple points using a digital micrometer and it was found to be 1 mm thick. The gel was kept in contact with 5 ml of phosphate buffer at pH 7.4 in the absence or in the presence of plasma proteins (albumin, γ-globulin, transferrin or fibrinogen) (concentration 10%, w/v). As a comparison the spectrum of each plasma protein in phosphate buffer solution at pH 7.4 and in the absence of was recorded.

3. Results and discussion

3.1. PHM synthesis

The partial derivatization of α,β-poly[*N*-(2-hydroxyethyl)-DL-aspartamide] (PHEA) with methacrylic anhydride (MA) allows to introduce in the side chain double bonds and ester groups, giving rise to the copolymer named PHM. Double bonds are reactive towards UV radiations chosen to crosslink PHM; whereas ester groups make potentially degradable the resulting PHM-based hydrogel.

In this work, a PHM copolymer with a derivatization degree of 30 ± 1 mol% has been prepared as previously reported (Mandracchia et al., 2004). Analytical and spectral data of PHM are in agreement with the attributed structure (Mandracchia et al., 2004).

3.2. Preparation of PHM-based hydrogel

An aqueous solutions of PHM has been irradiated, in the absence of photoinitiators at 313 nm for 3.5 h under argon to obtain the hydrogel schematically reported in Scheme 1.

The preparation of hydrogel without photoinitiators starting from PHM is possible thanks to the presence of several methacrylate residues in PHM structure that after UV irradiation give rise to a radical crosslinking. The absence of photoinitiators (such as benzophenone, acetophenone and 2,2-dimethoxy-2-phenylacetophenone) is important and advantageous since it is well known that they are reactive molecules usually dangerous for humans. As a consequence, hydrogels obtained without the use of photoinitiators are potentially biocompatible.

PHM-based hydrogel recovered after freeze-drying has been extensively characterized.

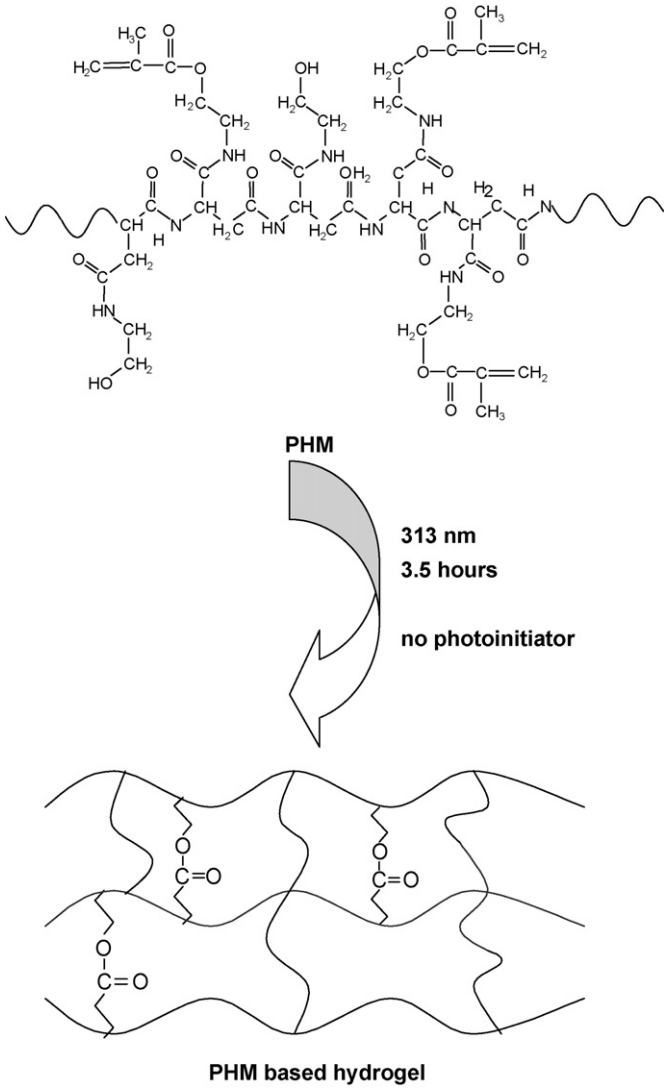
3.3. Characterization of PHM-based hydrogel

The occurrence of a chemical crosslinking has been confirmed by FT-IR analysis. In fact, FT-IR spectrum of PHM-based hydrogel shows the complete disappearance of the peaks at 1300 cm^{-1} (scissoring –C=**C**–**H**) and 950 cm^{-1} (wagging –C=**C**–**H**) that are showed in the spectrum of the starting PHM (see Fig. 2). This result confirms that the crosslinking reaction involves the double bonds of PHM, probably through the formation of free radicals that give rise to inter- and intra-polymeric carbon–carbon crosslinked bonds.

The physical state of PHM-based hydrogel has been determined by X-ray analysis; the X-ray diffraction patterns of uncrosslinked and crosslinked PHM (data not showed) evidence that both these samples are in an amorphous state, therefore, during the crosslinking reaction, no crystalline region was formed.

SEM micrography of PHM-based hydrogel, reported in Fig. 3, shows a porous surface of the hydrogel in agreement with the formation of a polymer network.

The presence of micropores could facilitate the interaction with the aqueous medium, potentially improving swelling ability of PHM-based hydrogel. On the other hand, biocompatibility, surface properties, rate of enzymatic or chemical degradation,



Scheme 1. Schematic representation of the formation of PHM-based hydrogel.

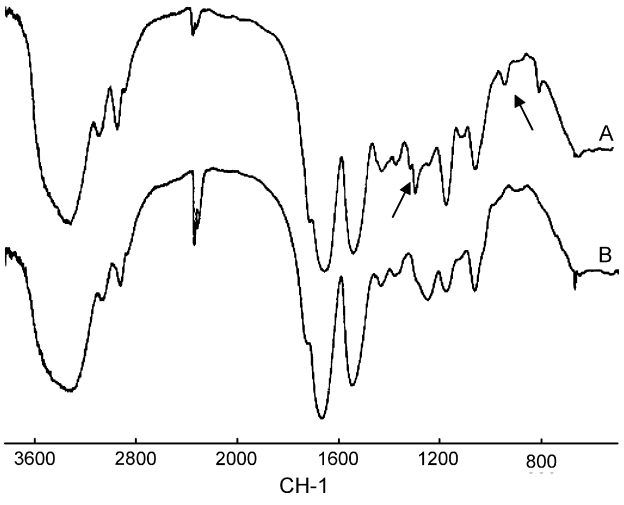


Fig. 2. FT-IR spectra of: (A) uncrosslinked PHM; (B) crosslinked PHM.

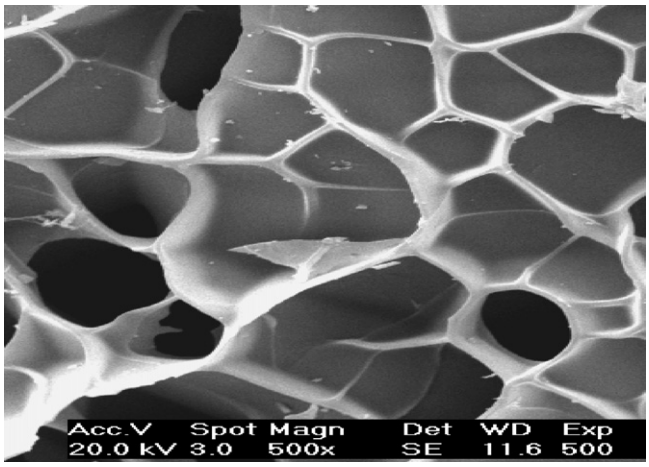


Fig. 3. SEM micrograph of outside surface of PHM-based hydrogel.

absorption and diffusion of solutes and mechanical characteristics strongly depend on the swelling behaviour. For these reasons, the weight swelling ratio, q, of the PHM-based hydrogel has been determined in twice-distilled water and in phosphate buffer solution at pH 7.4 as a function of time. Experimental data reported in Fig. 4 evidence a rapid swelling and a good affinity of the hydrogel towards the investigated media. The small difference found by varying the external medium, is probably due to the osmotic pressure in phosphate buffer solution at pH 7.4 that causes a weight swelling ratio lower than that found in twice-distilled water.

Owing to the presence of ester bonds potentially biodegradable by chemical and/or enzymatic hydrolysis, the PHM-based hydrogel has been incubated in phosphate buffer solution at pH 7.4 in the absence or in the presence of esterase until 20 days.

The yield of the hydrogel recovered as a function of the incubation time and its swelling ability (evaluated in twice-distilled water) have been taken as a measure of the occurred progressive degradation (see Figs. 5 and 6, respectively).

It is evident that PHM-based hydrogel undergoes a decrease in its weight whereas its swelling ability increases as a function of hydrolysis time, especially in the presence of esterase. Besides, at longer incubation times, the hydrolytic effect of esterase is more pronounced; this suggests a slow action of the

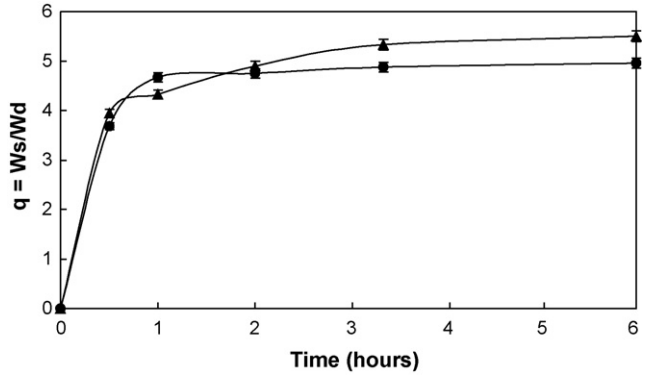


Fig. 4. Weight swelling ratio, q, of the PHM-based hydrogel determined in twice-distilled water (▲) and in phosphate buffer solution at pH 7.4 (●) as a function of time.

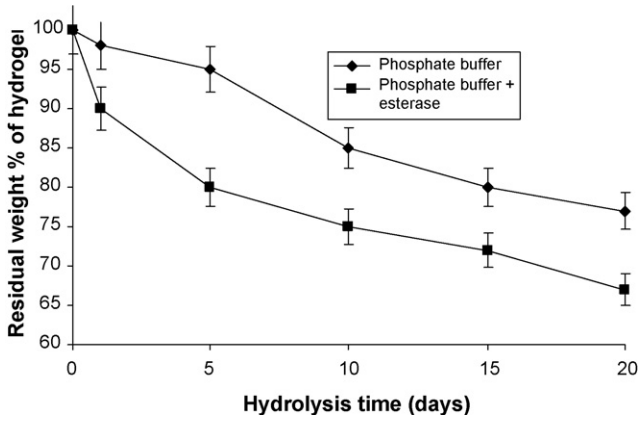


Fig. 5. Residual weight percentage of the PHM-based hydrogel recovered after incubation with phosphate buffer solution at pH 7.4 in the absence or in the presence of esterase (0.5 mg/ml) at 37 °C as a function of hydrolysis time.

enzymes that, probably, initially hydrolyze the external sites of the hydrogel, but, elapsing time, they penetrate into the polymer network hydrolyzing also internal sites. Obviously, the degradation of PHM-based hydrogel after hydrolysis of ester groups, affords the formation of the starting PHEA that is a biocompatible polymer whose renal excretion has been elsewhere reported (Rypacek et al., 1982; Caliceti et al., 2001). Therefore, potentially, the degradation of PHM-based hydrogel does not cause accumulation of fragments in the body. In addition, the slow degradation that PHM-based hydrogel undergoes, could be an advantageous property in designing, for example, implants based on this material.

3.4. In vitro biocompatibility studies

The biocompatibility of the prepared hydrogel has been evaluated *in vitro* either by indirect or direct method, using human chronic myelogenous leukaemia cells (K-562), chosen as a model cell line (see Section 2).

No significant difference has been found between the sample and the control in both the employed methods (data not showed). This result suggests that PHM-based hydrogel does not release, in the growth medium, substances (e.g. degradation products), which affect cell viability and it does not cause a decrease in cell viability after direct contact with the cells.

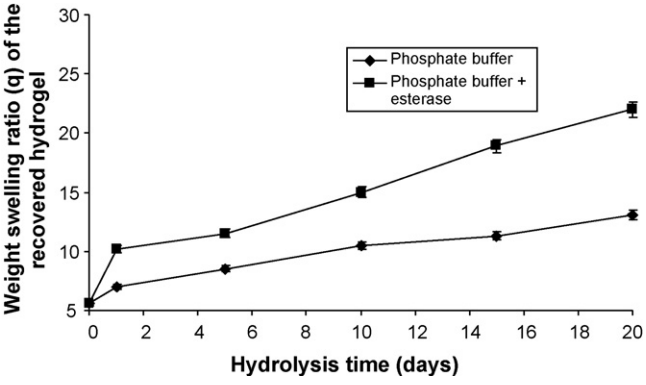


Fig. 6. Weight swelling ratio, q, of the PHM-based hydrogel recovered after incubation with phosphate buffer solution at pH 7.4 in the absence or in the presence of esterase (0.5 mg/ml) at 37 °C as a function of hydrolysis time. The value of q has been determined in twice-distilled water.

3.5. Interaction of PHM-based hydrogel with plasma proteins

It is well known that when an artificial surface is in contact with blood, a layer of adsorbed plasma proteins rapidly is formed on this surface (Van Damme, 1990). This phenomenon could cause conformational changes or denaturation of these proteins that jeopardize the clinical success of the artificial material employed for example for parenteral implant. More specifically the denaturation of adsorbed plasma proteins usually causes adhesion, aggregation and activation of platelets with blood clotting and thrombus formation.

Various authors have investigated the interaction of plasma proteins with different materials such as derivatives of polyaminoetherurethaneurea, poloxamer and poloxamine stabilized solid lipid nanoparticles, silica surfaces, polystyrene – graft – stearyl poly(ethylene oxide), poly(D,L-lactide-co-glycolide) or poly(L-lactide) (Sanada et al., 1986; Luck et al., 1998; Ji et al., 2000; Tarasevich and Monakhova, 2002; Goppert and Muller, 2005; Sibel et al., 2005). Among the new techniques that can be employed to study this phenomenon, the attenuated total reflection infrared spectroscopy (ATR-FTIR) has been successfully employed to evaluate, for example, the interaction between plasma proteins and crosslinked hyaluronan (Barbucci et al., 2002) or polysulfone (Bummer, 1996).

In this work, ATR-FTIR technique has been used to study the behaviour of four plasma proteins such as human albumin, γ-globulin, transferrin and fibrinogen when in contact with PHM-based hydrogel. All these proteins play important roles in the human organism, for example, albumin is necessary to regulate the blood osmotic pressure, γ-globulin is involved in the immune response, transferrin transports iron to cells and fibrinogen is essential for the coagulation process. Obviously, the evaluation of the interaction occurring between these plasma proteins and PHM-based hydrogel is of great interest with regard to its *in vivo* performance.

To perform ATR-FTIR analysis, the apparatus and the procedure reported in Section 2 have been employed. Fig. 7 shows

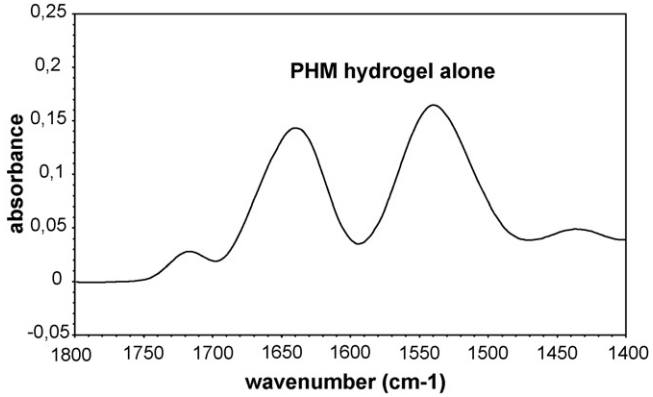


Fig. 7. ATR-FTR spectrum of PHM-based hydrogel alone.

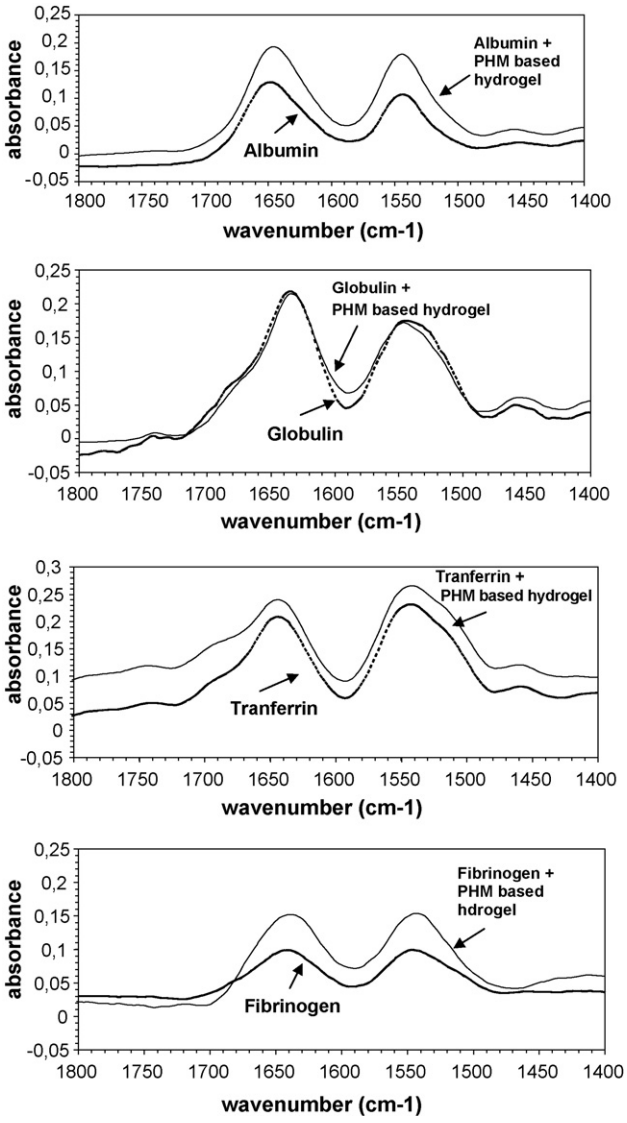


Fig. 8. ATR-FTIR spectrum of each investigated plasma protein alone or in the presence of PHM-based hydrogel.

the ATR-FTIR spectrum of PHM-based hydrogel alone, whereas Fig. 8 shows the spectrum of each plasma protein alone or in the presence of PHM-based hydrogel.

It is known that, from the analysis of the Amide I and Amide II bands, it is possible to obtain some information about the protein interaction with surfaces and, in particular, the protein conformation (such as α-helix and β-sheet structures) is related to the Amide I band structure.

Besides, it has been reported that protein adsorption strongly depends on the surface properties. In particular, hydrophobic character of a surface can be cause of interaction, adsorption and changes in conformation of plasma proteins, on the contrary a hydrophilic surface may be favourable for building up a hydration layer thus avoiding the adsorption and successively the denaturation of plasma proteins (Sanada et al., 1986; Barbucci et al., 2002). Since PHM-based hydrogel shows a remarkable water affinity, as confirmed by swelling measurements in aqueous media, it is reasonable to expect that the high amount of absorbed water prevents absorption and conformational changes of plasma proteins. On the contrary, if an interaction between plasma proteins and PHM-based hydrogel occurs, changes in the position and intensity of Amide I and Amide II bands should be observed.

It is evident from Fig. 8 that there are not significant variations in the Amide I and Amide II bands of each investigated protein when in contact with PHM-based hydrogel.

This result suggests that the investigated proteins do not interact significantly with the surface of PHM-based hydrogel. Although PHM-based hydrogel has a rather porous surface (as shown by scanning electron microscopy, see Fig. 3) that should lead to a larger surface area accessible for protein adsorption, probably, the high rate of swelling, the great amount of adsorbed water and the large protein size avoid the adsorption and the consequent conformational changes of proteins. This is an important result, i.e. the absence of interaction between plasma proteins and PHM-based hydrogel could ensure a potential blood compatibility of this hydrogel, that is one of the requirements for a parenteral system. All these considerations make PHM-based hydrogel an attractive material to prepare parenteral devices.

4. Conclusions

The present study deals with the preparation and characterization of a biodegradable and biocompatible hydrogel proposed as a new material for biomedical applications. The starting material has been a methacrylate derivative of the synthetic polymer α,β-poly(-*N*-2-hydroxyethyl)-DL-aspartamide (PHEA) obtained by derivatization of this polymer with methacrylic anhydride and named PHM. Aqueous solutions of PHM give rise to a chemical network after UV irradiation in the absence of toxic photoinitiators. PHM-based hydrogel shows interesting physicochemical properties such as a porous and amorphous structure, a rapid and remarkable swelling ability in simulated physiological medium and susceptibility of degradation, especially in the presence of esterase. Besides, this material and its degradation products do not cause a decrease in the cell viability of human chronic myelogenous leukaemia cells (K-562), chosen as a model cell line. Finally, ATR-FTIR spectroscopy demonstrated that the prepared hydrogel does not cause significant conformational changes in some plasma proteins, such as albumin, γ-globulin, transferrin and fibrinogen, thus indicating a potential blood compatibility of this hydrogel. All these properties make PHM-based hydrogel a potential candidate for preparing parenteral systems.

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