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Radio-synthesized polyacrylamide hydrogels for proteins release

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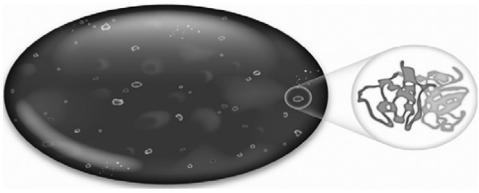
HIGHLIGHTS

- Method for synthesis of polyacrylamide (copolymer) hydrogels using γ-irradiation.
- Polyacrylamide hydrogels suitable for protein loading and release.
- Controlled release of proteins and bioactivity maintenance.
- Noncytotoxic profile observed for these protein containing hydrogels.

GRAPHICAL ABSTRACT



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ABSTRACT

The use of hydrogels for biomedical purposes has been extensively investigated. Pharmaceutical proteins correspond to highly active substances which may be applied for distinct purposes. This work concerns the development of radio-synthesized hydrogel for protein release, using papain and bovine serum albumin as model proteins. The polymer was solubilized (1% w/v) in water and lyophilized. The proteins were incorporated into the lyophilized polymer and the hydrogels were produced by simultaneous crosslinking and sterilization using γ-radiation under frozen conditions. The produced systems were characterized in terms of swelling degree, gel fraction, crosslinking density and evaluated according to protein release, bioactivity and cytotoxicity. The hydrogels developed presented different properties as a function of polymer concentration and the optimized results were found for the samples containing 4–5% (w/v) polyacrylamide. Protein release was controlled by the electrostatic affinity of acrylic moieties and proteins. This selection was based on the release of the proteins during the experiment period (up to 50 h), maintenance of enzyme activity and the nanostructure developed. The system was suitable for protein loading and release and according to the cytotoxic assay it was also adequate for biomedical purposes, however this method was not able to generate a matrix with controlled pore sizes.

1. Introduction

Hydrogel systems may be nanostructured by controlling pore size (Pacios et al., 2006) in order to achieve desirable physico-chemical and mechanical characteristics and also release profiles providing an optimized environment for such substances.

Polymers derivate from acrylates, vinyl alcohol, vinylpyrrolidone and specially acrylamide are currently selected for hydrogel synthesis mainly due their chemical properties, biocompatibility and low toxicicity being suitable to constitute pharmaceutical delivery systems (Rosiak et al., 1983; Rosiak and Ulanski, 1999; Peppas et al., 2000).

The selection of papain and BSA as a model proteins was based on well described structure for BSA and properties of wound debridement for papain (Gurung and Skalko-Basnet, 2009; Naddaf et al., 2010). Polyacrylamide was chosen due to its superabsorbent properties and its recognized applicability in biochemical processes (Bardajee et al., 2008).

Therapeutic proteins and proteolytic enzymes are currently loaded in hydrogels for wound treatment. Although these products have shown efficacy, problems regarding stability of the biomolecule in the pharmaceutical form restricts its use.

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This work aimed the synthesis of a nanostructured PAAM membrane suitable for protein release crosslinked by ionizing radiation using papain and bovine serum albumin (BSA) as protein models in order to evaluate the influence of pore size in release rate and the enzyme stability in this encapsulating system.

2. Experimental

2.1. Materials

Commercially available non-ionic grade polyacrylamide (PAAM) (molecular weight 2.5×10^7 g mol^{-1}) was obtained from Produquímica (Brazil) with 30% of hydrolysis (copolymer of acrylamide and acrylic acid); Papain (30,000 USP ml^{-1}) was purchased from Merck (Brazil); Bovine Serum Albumin (BSA) and *n*-benzoyl-DL-arginine-*p*-nitroanilide was purchased from Sigma Aldrich (Germany). Milli *Q*™ water was used in all experiments. All chemicals were of analytical grade.

2.2. Methods

2.2.1. Hydrogel synthesis

Sample of PAAM was solubilized in water to reach a polymer concentration of 1% (w/v) and then submitted to freezing drying process. Aliquots of water (control membranes) or protein solution were added to lyophilized powder to reach final polymer concentrations of 4, 5, 6 and 10% (w/v). Radiation induced simultaneous crosslinking and sterilization (Rosiak and Olejniczak, 1993) using 25 kGy at dose rate of 1.4 kGy h^{-1} was used for hydrogels synthesis. All samples were frozen at −20 °C and irradiated in the presence of solid carbon dioxide.

2.2.2. Protein loading

Papain and BSA solutions were added before irradiation process under the same conditions adopted for the control membranes to reach final concentration of 0.2% (w/v) to papain and 1% (w/v) to BSA.

2.3. Hydrogel characterization

2.3.1. Swelling degree

Samples of PAAM hydrogels (aprox. 1 g) were oven dried until constant weight. The samples were immersed in physiological solution in excess, to remove the uncrosslinked fractions, and at different time intervals the weight was registered until equilibrium was established (constant weight). The swelling equilibrium was calculated according to the following equation

$$S = (W_f - W_i)/W_i \; 100, \tag{1}$$

where W_i is the initial weight (dried sample) and W_f is the Swollen weight (dried sample).

2.3.2. Crosslinking density determination

Crosslinking density was determined according to Flory (1953), Rosiak et al. (1988), Mahmudi et al. (2007). The following equations

$$M_c = -\ln(1-\chi) + \chi + \mu\chi^2 + p \times v/(\chi^{1/3} - 0.5\chi) \tag{2}$$

$$q = w/M_c \tag{3}$$

were applied.

The parameters selected corresponded to $p = 0.77$ g mol^{-1} and $v = 18$ g mol^{-1}. The polymer/solvent interaction parameter $\mu = 0.495$ was based on Mark (1999), χ value was obtained for each sample based in swollen hydrogel data, $w = 71$ g mol^{-1} and q corresponded to the crosslinking density values (g cm^{-3}).

2.4. Biological approach

2.4.1. Protein release

Protein release was measured by immersion of the matrices in buffer physiological solution pH 6.0 at 37 °C. Aliquots (2 ml) were collected as a function of time (0–50 h) and characterized in terms of protein content for both proteins and bioactivity for papain.

2.4.2. Protein content determination

Drug-loaded hydrogels were immersed in phosphate buffer pH 6.0 and the aliquots were taken and measured by UV–vis spectrophotometry ($\lambda = 280$ nm) on a Hitachi spectrophotometer model Cary 1E.

2.4.3. Enzymatic activity assay

Papain activity was measurement using *n*-benzoyl-DL-arginine-*p*-nitroanilide (Erlanger et al., 1961) as a substrate at 40 °C using phosphate buffer pH 6.0 containing cysteine on a microplate and assayed on a Elisa reader model Multiskan EX Microplate Photometer Thermo Scientific ($\lambda = 405$ nm).

2.4.4. Citotoxicity

Balb 3T3 cells (CCL-163™; ATCC, Manassas, VA, USA) were cultured at 37 °C in 5% (v/v) CO_2 and 97% humidity in complete DMEM tissue culture medium supplemented with 10% (v/v) fetal bovine serum, 100 IU penicillin/100 μg ml^{-1} streptomycin and 4 mM L-glutamine. The assay was conducted according to Esteves-Pedro et al. (2011). Briefly the effects of membrane extract (ISO 10993/EN 30993, 1992) on cell proliferation were added to 96-well plates containing 15,000 cells, followed by addition of eight different concentrations of membrane extract (100 to 0.8% (v/v)). The cytotoxicity evaluation was carried out by using the CellTiter 96R Aqueous Non-radioactive Cell Proliferation Assay and the amount of formazan produced by the cells was determined by measuring sample absorbance at 490 nm with a spectrophotometer (SpectraMaxR 190—molecular devices).

3. Results and discussion

3.1. Hydrogel synthesis

The PAAM hydrogels were prepared by freeze-drying in order to allow a much higher proximity of polymer molecules since this condition promotes polymer crosslinking. Moreover, low temperatures are suitable to encapsulate proteins since under low temperatures protein degradation is minimized, favoring protein stability and integrity.

Radiation induces chain scission and crosslinking depending among other factors on the temperature and chemical structure (Schnabel, 1981). Under low temperatures, as reported by Ozmen et al. (2007) chain scission and radiation indirect effects—water radiolysis were impaired. Regarding the synthesis of PAAM hydrogels, the samples irradiated at room temperature showed no membrane formation; on the other hand it showed an intense decrease in viscosity. This was an evidence of predominance of chain scission over crosslinking. Such fact was not observed for the samples irradiated under low temperatures, where membrane formation occurred. Under freezing conditions the polymer molecules were excluded from ice crystals, therefore they were in close contact each other. Under this special condition, the crosslinking of PAAM solutions were achieved and these membranes were then submitted to characterization.