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Designing novel macroporous composite hydrogels based on methacrylic acid copolymers and chitosan and *in vitro* assessment of lysozyme controlled delivery

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ABSTRACT

Designing structure and morphology of macroporous hydrogels is crucial for their applications in controlled release systems of macromolecular drugs. Macroporous hydrogels, consisting of methacrylic acid (MAA) and either acryl amide (AAm) or 2-hydroxyethyl methacrylate (HEMA) (1st network), were prepared for this purpose by cryogelation (single network cryogels, SNCs). Macroporous interpenetrating polymer network (IPN) hydrogel composites were then prepared by a sequential strategy, the 2nd network consisting of chitosan (CS) cross-linked with poly(ethyleneglycol) diglycidyl ether (PEGDGE) being generated by the sorption of a CS and PEGDGE mixture in the 1st network followed by cross-linking. A strong difference in the behavior of SNCs and IPN hydrogel composites was found during the loading and release of lysozyme (LYS) used as macromolecular drug model. Thus, while the amount of LYS loaded on SNCs was higher than that loaded on the IPNs, the release of LYS from SNCs occurred at pH 2, when the ratio between MAA and AAm was 50:50, and only at pH 1 when the ratio between MAA and AAm was 70:30. The 2nd network led to the decrease of the pore size of the IPNs, mainly when the initial concentration of monomers was 10 wt/v%, but the presence of CS facilitates the LYS release from IPNs, mainly at a concentration of monomer of 5 wt/v%, and when HEMA was used as nonionic comonomer.

1. Introduction

Hydrogels are polymeric networks capable to retain large amounts of water, or biological fluids, which have a soft and rubbery consistence being thus similar with living tissues [1]. Due to their particular properties, hydrogels have various biomedical applications, such as: soft contact lenses, tissue engineering scaffolds, wound dressing, mechanical actuators, biosensors, bioseparation, drug delivery systems (DDS), *etc.* [1–4]. Among ionic hydrogels, those based on methacrylic acid (MAA) have been intensively used for various applications such as drug delivery and separation of ionic species [5–7]. However, the poor mechanical strength, the slow response at stimuli as well as the collapse under strong acidic conditions of the conventional single-network PMAA hydrogels constitute technical drawbacks for their applications [8]. By the introduction of additional comonomers in the polymer chain more widely controllable physical properties, and, frequently, more efficient drug loading/release properties have been induced [8,9]. Interpenetrating polymer networks (IPN) have been widely used last decade to generate more sophisticated and more efficient DDSs [10–17] as well as robust scaffolds for cell proliferation in regenerative medicine applications [18,19]. Among the IPN composite hydrogels for biomedical applications, those composed of PMAA and polysaccharides are of large interest. Thus, composite hydrogels composed of chitosan (CS) and PMAA or the copolymers of MAA or acrylic acid (AA) with acrylamide (AAm) or other acids, have been synthesized and characterized [11,20–23], their applications mainly in DDSs being evaluated [23–25]. Semi-IPN hydrogels have been synthesized either by including CS in the initial mixture of acrylic monomers, or by mixing CS with preformed homopolymers (PMAA or PAA), followed by the selective cross-linking of CS mainly with dialdehydes [26,27].

It is known that the therapeutic protein administration usually asks for frequent doses due to the small residence of protein in blood [21]. Therefore, the development of novel sustained drug release systems would help to overcome this drawback [14,16,28–30]. Keeping in mind the potential of PMAA/CS composite hydrogels for controlled DDSs, a novel strategy was adopted

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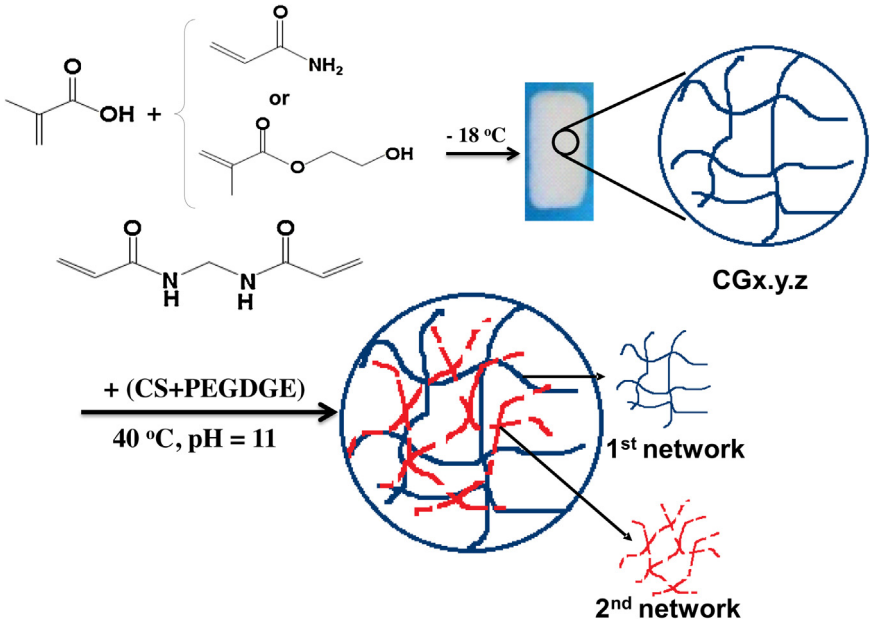

Scheme 1. Synthesis strategy of the IPNs hydrogel composites. The 1st network is called CG*x.y.z* to show that cryogels with various ratios between monomers (*x*), cross-linking ratio (*y*), and initial monomer concentrations (*z*) were prepared.

in this work for the preparation of full-IPN P(MAA-*co*-AAm)/CS and P[(MAA-*co*-2-hydroxyethylmethacrylate (HEMA))]/CS hydrogel composites by the sequential technique. The 1st network consisting of P(MAA-*co*-AAm) or P(MAA-*co*-HEMA) cryogel, was synthesized by cryogelation at −18 °C and the 2nd network was generated by an amine/epoxy addition reaction between the primary amine groups of CS and the epoxy groups in poly(ethyleneglycol) diglycidyl ether (PEGDGE), in basic medium. Advantages of cryogelation consist of the absence of any organic or inorganic porogen, the ice crystals playing the role of inert template, the microstructure of the gel being the negative replica of the ice crystals [31–35]. Cryogels are endowed with a capillary network and a high osmotic stability, which make them adequate materials for various biomedical applications and separations [36,37]. Single network cryogels (SNCs) and IPN composite gels were characterized by Fourier transform infrared (FT-IR) spectroscopy, scanning electron microscopy (SEM), thermogravimetry, swelling kinetics, and equilibrium swelling as a function of pH. Loading of the SNCs and of the IPN composite gels with lysozyme (LYS) and the controlled protein release were investigated as a function of the gel composition and the environment pH. Due to its antibacterial activity against Gram-positive bacteria and the potential to be used as an anticancer drug, LYS is used in food processing, and pharmaceutical industry, numerous studies being focused on the LYS purification [38] and on the controlled release of LYS from various materials [39–41]. Even if the study of the swelling behavior and biomedical applications of the MAA based hydrogels attracted large of attention, the synthesis, and the potential in the controlled release of LYS from the MAA based cryogels and from their sequential IPNs with CS, as a function of the gel composition, have never been investigated up to now.

2. Materials and methods

2.1. Materials

MAA and HEMA, purchased from Sigma–Aldrich, were distilled under reduced pressure before use and stored at 4 °C. AAm, from Fluka, *N*,*N*′-methylenebisacrylamide (BAAm), ammonium persulfate (APS), *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED), and PEGDGE with average molar mass M_n of 526 g/mol, all from Sigma–Aldrich, were used as received. CS as powder with molar mass of 467 kDa and an average degree of acetylation = 15% [42], purchased from Sigma–Aldrich, was used as received. Solution of CS with a concentration of 2 wt.% was obtained by dissolving the CS powder in 1 v/v% acetic acid aqueous solution and moderate stirring for 24 h. LYS from chicken egg white, with a molar mass of 14,400 g/mol, was purchased from Fluka and used without further purification.

2.2. Preparation of SNCs and IPN hydrogel composites

SNCs were prepared by free-radical cryopolymerization of MAA sodium salt with either AAm or HEMA as comonomers, at −18 °C. The redox initiator system used consisted of APS and TEMED. The general code of the SNCs consists of "A" for P(MAA-*co*-AAm) and "H" for P(MAA-*co*-HEMA), followed by three numbers separated by dots: the first one represents the mole ratio between MAA and the second comonomer, the second one represents the mole number of monomers (MAA + AAm or HEMA) for 1 mol of BAAm (cross-linker ratio), and the third one represents the total initial concentration of monomers expressed in wt/v%. The preparation of SNC A73.20.10, where 73 means 70 mol% of MAA and 30 mol% of AAm, 20 represents 20 monomer units for one mole of BAAm, and 10 shows the initial monomer concentration in wt/v%, is presented below as an example. Typically, 0.2386 g AAm, 0.665 mL MAA, 3.45 mL BAAm (0.625 g/25 mL) and 0.5 mL TEMED (1.0 mL/25 mL) stock solutions, were first mixed in a flask of 10 mL, the solution pH being adjusted at 7–8 with aqueous solution of NaOH 5 M, and the volume of solution was adjusted at 9 mL. The solution was cooled at 0 °C in ice–water bath, purged with nitrogen gas for 20 min and then, 1 mL of cold APS stock solution (0.5 g/25 mL) was added, the mixture being well homogenized about 20 s. Portions of this solution, each 2 mL, were transferred to syringes of 8 mm in diameter; the syringes were sealed, and immersed in a cryostat at −18 °C for one day. After thawing, the gels were cut into specimens of approximately 10 mm in length and immersed in distilled water, changing water each 4 h to wash out any unreacted monomers and the initiator, about one