pH-responsive casein-based films and their application as functional coatings in solid dosage formulations

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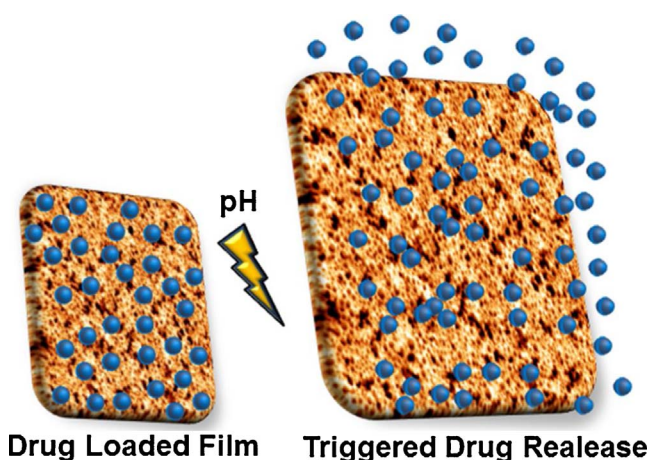[c] Departamento de Farmacia, Facultad de Ciencias Químicas (Universidad Nacional de Córdoba), UNITEFA-CONICET, Haya de la Torre y Medina Allende, Córdoba X5000HUA, Argentina
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GRAPHICAL ABSTRACT

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ABSTRACT

Over the past few years, there has been increasing attention to the design of new drug delivery systems based on milk proteins due to their excellent functional properties. In this study, we report a new approach for preparing protein-based coating films using highly methacrylated casein as a macro-crosslinker. The films exhibited a smooth surface and an even casein distribution as evidenced by both scanning electron and atomic force microscopy, respectively. The mechanical behavior of the casein-based films could be easily tuned by controlling the degree of methacrylation of the bio-based crosslinker. The as-prepared films showed pH-dependent swelling, in which the swelling ability in alkaline medium was stronger than that at acid pH. The mechanical, swelling,

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rheological and release performance of the casein-based films suggest that these materials have a high potential as enteric coatings for intestinal drug delivery.

1. Introduction

The use of bio-based polymers to prepare pharmaceuticals and biomedical agents has generated considerable research interest in recent years regarding relieving the overdependence on synthetic polymeric materials [1–4]. In addition, recent advances in the design of polymer-based stimuli-responsive systems have created opportunities for novel applications in different fields such as smart coating [5], biosensors [6] optical materials [7,8] and drug delivery [9], among others. Currently, controlled drug delivery systems represent the main biomedical application for biopolymers [10,11]. Control of the stability and release profiles of pharmaceutical drugs are topics that are of special interest in the field of delivery technologies. Thus, new materials with innovative functional properties are continually undergoing development. In this scenario, stimuli-sensitive polymers could be considered as the new generation of functional materials for biomedical applications [12].

In the last few years, strict health regulations and consumer preferences have driven the use of natural polymers due to their recognized/known non-toxicity as vehicles [13]. In this context, polypeptides and proteins are a promising alternative as building blocks of novel drug delivery systems, due to their excellent functional properties, high biocompatibility and easy degradation by enzymatic action under physiological conditions [14–16]. Among the different commercially available proteins, casein has gained notoriety due to its structural and physicochemical properties that facilitate its use in drug delivery systems [17]. Relevant characteristics of casein such as non-toxicity, high thermal stability, biocompatibility, biodegradability, ability to bind ions and small molecules, outstanding gelation and water binding capacities, and micelle formation capability, make this protein a highly attractive option [18]. Moreover, the amino acid composition in casein confers it a pH-responsive behavior, which could be used to design programmable-release materials. Several techniques have been proposed to design casein-based carriers in the form of composites, hydrogels, beads, and micro- or nano-particles [3].

So far, the use of casein to produce coatings for pharmaceutical solid dosage has hardly been explored. Abu Diak et al. [19] reported the use of this biopolymer as a film- former for tablet coating. In this study, different plasticizing agents such as glycerol, triethyl citrate, dibutyl sebacate and oleic acid were evaluated to produce casein films covering diltiazem HCl core tablets. The results showed that only oleic acid was able to produce smooth coats. Although casein represents a feasible alternative to obtain commonly used film coats, the major drawback for its widespread use is its limited mechanical strength. Thus, several investigations have focused on the use of crosslinkers to modify its mechanical properties [20]. In view of this, acrylic/casein hybrid materials appear as an excellent alternative due to their improved mechanical properties [21–23]. For instance, Ma et al. [2] reported the synthesis of casein-based silica nanocomposite films as a drug carrier. These films were found to be pH-responsive, and the silica content was proved to have positive effects on both the drug-loading capacity and drug-releasing behavior. Recently, a novel strategy for synthesizing acrylic/casein film-forming dispersions with an appropriate control of synthetic/biopolymer compatibility was proposed [22–24]. The synthesis method involves the use of highly methacrylated casein acting as a macro-crosslinker in an emulsion polymerization of acrylic monomers, with the aim of promoting polymer grafting onto the protein backbone. Film-forming dispersions are widely used in the pharmaceutical industry and are mostly applied over solid dosage forms (tablets, capsules, microparticles, etc.) for the development of coatings with

different functionalities. Thus, functional coatings are used for the improvement of organoleptic features, protection of the gastrointestinal tract (GIT) from the irritant effect of certain drugs, prevention of drug degradation in the GIT environment (pH conditions or presence of enzymes) and, more importantly, for the achievement of site-specific and controlled release drug delivery [25].

In this context, the use of casein macro-crosslinker (CMC) to prepare film-forming dispersions could improve the protein incorporation in the final materials, which in turn would enhance their pH responsiveness as functional coating. For this reason, in this work we propose for the first time the synthesis of unique film-forming dispersions by using derivative casein for smart drug delivery systems. The degree of functionality of the CMC was varied to tune the physicochemical properties of the resulting hybrid polymers. Finally, the targeted application of the obtained casein-based films as functional carriers was evaluated using bovine serum albumin and ofloxacin as model drugs.

2. Experimental

2.1. Materials

Technical grade casein from bovine milk (Sigma), methyl methacrylate (MMA), butyl acrylate (BA) and glycidyl methacrylate (GMA) (Aldrich) were used as supplied. The initiator used was tert-butyl hydroperoxide (TBHP, Aldrich). Buffers of pH 2.0 [hydrochloric acid (Cicarelli)/potassium hydrogen phthalate (Anedra)], pH 7.4 (sodium dihydrogen phosphate (Biopack)/disodium hydrogen phosphate (Anedra)] and pH 10.0 [sodium carbonate (Anedra)/sodium hydrogen carbonate (Cicarelli)] were prepared using deionized water. Phosphotungstic acid (PTA, Fluka) and Formvar® (polyvinyl formal, Fluka) were used for transmission electron microscopy (TEM) sample preparation. Bovine serum albumin (BSA, Sigma) and ofloxacin (OFL, Parafarm) were used for in vitro release studies. All the reagents were used as received without any purification. In the experiments, distilled water was used.

2.2. Synthesis of casein-based film-forming dispersions

Poly(BA-co-MMA)/casein dispersions were synthesized in batch by emulsifier-free emulsion polymerization using methacrylated casein as a macro-crosslinker and TBHP as an initiator. Synthesis details were previously described [22]. The solid content was 20 wt% and the BA/MMA weight ratio was 80/20 in all cases. This formulation was used to obtain a copolymer with a glass transition temperature (T_g) suitable to be used as film coats. Four CMC with different degrees of methacrylation (10, 20, 30 and 40 theoretical vinyl bonds per molecule) were successfully prepared using GMA as a functionalizing agent according to a recently reported procedure elsewhere [22] and schematized in Fig. 1. The yield of the functionalization reaction was determined by ^{1}H-NMR using a Bruker Advance III 700 spectrometer. The area of the vinyl proton signals at 5.7 and 6.1 ppm of dialyzed samples at the end of the reaction (where unreacted GMA was removed) was compared with that corresponding to non-purified samples, wherein the total vinyl bonds of GMA were present. Thus, the ratio of both areas was used for estimating the yield of functionalization. In the calculation, unmodified casein signal at 0.75 ppm was taken as a reference. Yield values of 40, 35 and 25% were obtained for CMC with 10, 20 and 40 vinyl bonds per molecule, respectively. A detailed spectroscopy characterization of the CMC can be found in Fig. S1 of the Supplementary material (SM).
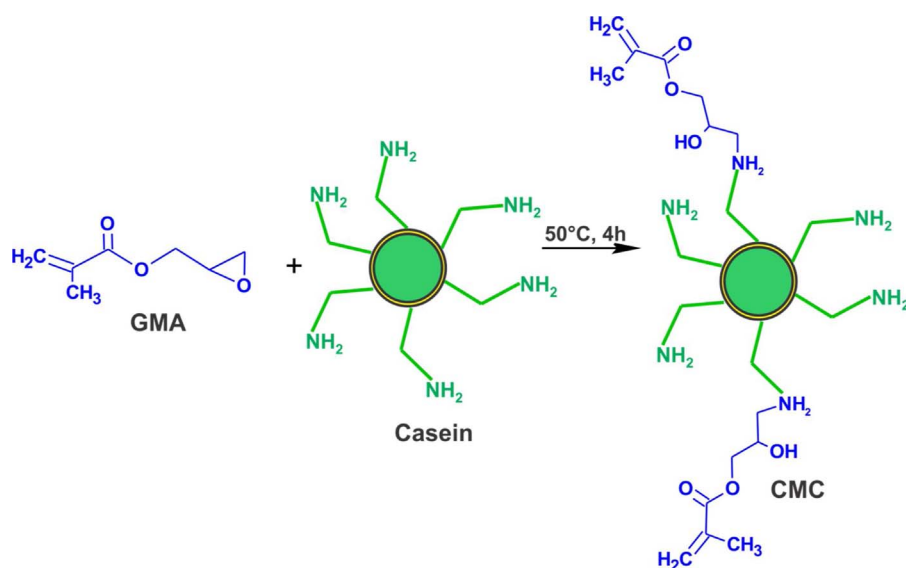
2.3. Characterization of the casein-based film-forming dispersions

Z-average particle size (Dp) was measured by dynamic light scattering on a Zetasizer Nano ZS (Malvern Instruments). Measurements were carried out at a scattering angle of 173° and a laser wavelength of 633 nm. Measurements were taken in triplicate measured at 25 °C. The number of particles per liter of latex (Np) was estimated from measurements of Dp and latex conversion. Capillary hydrodynamic fractionation (CHDF2000 Matec Applied Sciences) was performed to determine the particle size distribution (PSD) of the dispersions. Samples were prepared with deionized water at a concentration of 1.5% of solid content.

The viscosity (η) vs. shear rate (γ̇) profiles of the dispersions were obtained using a rheometer Anton Paar Physica MCR 301 model at 20 °C in a shear rate range of 0.1–10,000 s^{-1}. A cone–plate geometry of 50 mm and a gap of 0.05 mm were used.

UV spectroscopy (Perkin Elmer Lambda 25 spectrometer) was employed to determine the consumption of bio-crosslinker. To this effect, the unreacted CMC was extracted from the samples by multiple centrifugation and redispersion steps, and quantified by using a calibration curve with neat casein in water ((λ = 280 nm). The CMC consumption was then expressed as the ratio between the reacted and initially loaded protein [26].

Furthermore, the compatibility of latex particles was evaluated by TEM, using a JEM 2100F (200kV). A drop of diluted latex (0.01 wt% of solid content) was dried on a Formvar® coated copper grid. Then, a drop of 1 wt% PTA solution was added to stain the surface particles negatively [27].

2.4. Characterization of the casein-based films

Films with a final thickness of about 1 mm were prepared by casting the dispersions onto silicone molds (14 × 6 cm) and then dried at room temperature for seven days.

The film surface morphology of the casein-based films was determined by Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM). For SEM (Zeiss Sigma) analysis, films were coated with gold in a sputter coater and observed under an accelerating voltage of 2.0 kV. AFM (Nanotec Electronic) analysis was performed in tapping mode using In-One-Al silicon cantilevers (Budget Sensors) with a nominal spring constant of k = 40 N/m and a resonance frequency of 350 kHz. For the test, samples were cast onto seal paper (120 μm wet-thickness) and dried at room temperature overnight. WS × M free software was used to acquire and process the film images [28].

Stress–strain experiments were performed on a universal testing machine (INSTRON 3344) following the standard test method ASTM D882. Films with dog bone shape (length 9.53 mm and cross-section 3.18 × 1 mm^2) were tested at a rate of grip separation of 25 mm/min and 23 °C. Five specimens of each sample were analyzed and the average values are reported.

2.5. pH-response swelling studies

For studying the film's swelling response to pH changes, dry discs of 10 mm in diameter were immersed in buffers of pH 2, 7.4, and 10 at 25 °C. The pH values of the buffers were chosen taking into account the isoelectric point (IP, approximately of 4.6) of the casein. The discs were then removed from the medium at regular time intervals, weighed, and immersed again into the buffer. This procedure was repeated for seven days in order to ensure an equilibrium swelling. The degree of swelling at different times (DS$_t$) and at the equilibrium (DS$_e$) were determined according to Eqs. (1) and (2) [29], where W_t, W_e and W_d are the weight of the swollen sample at time t, the swollen sample at the equilibrium and the dry sample, respectively.

$$DS_t = (W_t - W_d)/W_d \tag{1}$$

$$DS_e = (W_e - W_d)/W_d \tag{2}$$

2.6. Rheological characterization

Rheological characterization was carried out with a rotational rheometer Anton Paar Physica, MCR 301 model.

The dynamic viscoelastic behavior of the films was studied in shear using plate–plate geometry (8 mm). Film discs of 8 mm in diameter and 1 mm in thickness were prepared. The temperature was varied from 130 to 0 °C with a cooling rate of 5 °C/min; and the frequency, deformation and normal force were fixed at 1 Hz, 0.1% and 4 N, respectively.

Rheological studies were also carried out on swollen samples for 24 h in simulated gastric fluid (SGF, pH 1.2) or phosphate buffered saline (PBS, pH 6.8). Experiments were conducted at a fixed strain of 1%, with a frequency sweep from 0.1 to 100 Hz at 20 °C. Plate–plate geometry (8 mm) was used to test swollen discs of 8 mm in diameter and 1 mm in thickness. Prior to the measurements, the linear viscoelastic region (LVR) of each material was determined through amplitude sweep studies. Strain was varied from 0.1 to 20% at a constant angular frequency of 10 Hz and 20 °C.

2.7. In vitro drug delivery studies

Release studies were conducted employing BSA and OFL as model drugs in order to analyze the potential use of the films in the design of an oral drug delivery system. For BSA loading, 800 mg of protein were dissolved in PBS (1 mL), and then mixed with 10 mL of latex. When employing OFL as a model drug, 500 mg were dispersed in 10 mL of latex, by using a high speed homogenizer Ultraturrax T18° IKA, (22000 rpm, for 1 min). Films of 1 mm in thickness were formed by casting loaded dispersions in silicone molds (4 × 4 cm) at room temperature for 7 days. Then film discs of 10 mm in diameter containing the drug were cut. Finally, the amount of drug loaded in each disc was estimated from the initial amount of drug dispersed in the latex and the weight of the loaded dry film.

A Sotax Smart AT7 dissolution equipment was used to investigate the release behavior of BSA or OFL from the loaded casein-based films. The test was performed on an Apparatus 1 (basket) according to US Pharmacopeia [30], at 50 rpm and 37 °C. Drug-loaded dry discs (around 25 mg of active ingredient) were immersed in a recipient containing 500 mL of buffer (pH 6.8 or pH 1.2) in order to ensure sink conditions. Samples (4 mL) were taken along the test at different time intervals, followed by adding an equal volume of fresh buffer into the receptor media. The amount of BSA or OFL released was determined spectrophotometrically, employing a calibration curve for each medium (at 280 and 294 nm, respectively). Average values are reported from duplicate measurements.

3. Results and discussion

3.1. Synthesis of the casein-based film-forming dispersion

Casein-based dispersions were identified as CBD_{10}, CBD_{20}, CBD_{30} and CBD_{40} according to the macro-crosslinker functionalities employed in the synthesis. From Fig. 2, it can be seen that increases in the number of methacrylic groups onto the CMC led to bigger particles and higher polydispersity index (see Fig. S2 of the SM), possibly due to limitations in the initiation of the polymerization process [21]. Through polymerization, radicals were generated by the redox reaction between the TBHP and amine groups of casein, which were considerably reduced when the methacrylation was increased (Fig. 1). As a consequence, the concentration of free radicals decreased, restricting particle nucleation (Np calculated from Dp data was found to decrease from 3.28×10^{16} for CBD_{10} to 1.05×10^{16} for CBD_{40}). On the other hand, it is worth noting that the fraction of CMC consumed was notably increased from 34 to 76% for CBD_{10} and CBD_{40}, respectively. This result shows that the degree of methacrylation of the macro-crosslinker allowed a fine control over materials compatibility.

Fig. 3a shows the PSD of the final dispersions (CBD_{10}, CBD_{20} and CBD_{40}). Note that when the number of functionalities used was 10 and
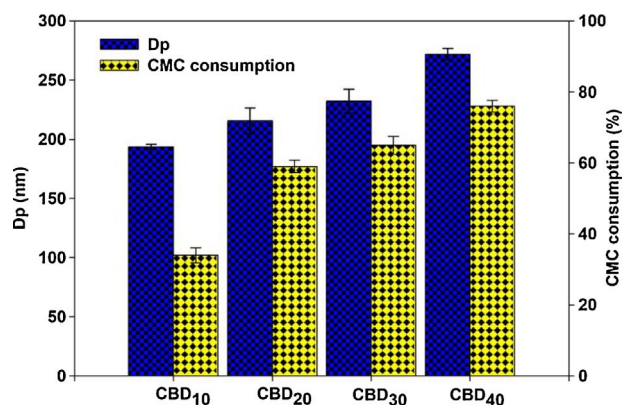
20, bimodal dispersions with a particle population at small size (around 50 nm) were produced, probably formed by micellar and/or homogeneous mechanisms [26]. Curiously, the use of a higher level of methacrylation in CBD_{40} led to the formation of a third population of large particles with a maximum size close to 270 nm. This high-size mode may correspond to particle associations that were probably generated by interparticulate crosslinking as a consequence of the large number of vinyl functionalities available in the casein. This hypothesis is supported by the final particle morphology of CBD_{40} observed by TEM.

The TEM micrograph of Fig. 3b for CBD_{40} presents an uneven PSD with the presence of both individual small particles and large ones covered by a dark shell that corresponds to the casein stained with PTA. In Fig. S4 of the SM, large cover-casein particles are shown in detail. From a TEM micrograph with a higher magnification (Fig. 3c) it can be perceived that the large dark particles actually consist of agglutinated smaller particles joined by a casein shell. These results clearly demonstrate that interparticle crosslinking could occur during polymerization with high levels of casein methacrylation. Practical experience has shown that dispersions of large particles are less viscous than those of smaller ones. Probably, for a given solid content, dispersion with larger particles presents a lower particle concentration, and hence, a lesser effect of interparticle interaction, since the distance between particles is higher in relation to that of the dispersion with smaller particles [31]. Following this reasoning, the viscosity of the dispersions should decrease with the degree of casein methacrylation. Fig. 3d shows viscosity curves for casein-based dispersions. It can be observed that all the curves displayed a Newtonian behavior at low shear rates and shear thinning at intermediate rates, which is typical for polymer latex. Moreover, note that, contrary to expectations, for degrees of methacrylation of 30 and 40 (for CBD_{30} and CBD_{40}, respectively), an important increase in dispersion viscosity at low rates was evidenced, suggesting that interparticle crosslinks play a key role in the rheological behavior of hybrid systems. Aggregation of polymer particles increased the dispersion viscosity by trapping water between particles clusters and thus producing an increment in solid content by transferring volume from the aqueous phase to the dispersed phase [32]. Viscosity is a sensitive parameter regarding the potential use of these dispersions in pharmaceutical products as coating agents for controlled-release formulations. The coating of tablets at the industrial scale is usually performed in coating pans while multiparticulate systems (pellets, microparticles, etc.) are processed via fluidized beds [33]. In both cases, the liquid coating agent is sprayed over the formulation surface and solvent evaporation occurs by means of circulation of air with controlled temperature. Thus, the degree of functionalization of CMC must ensure a correct balance between low viscosity and high compatibility in order to achieve trouble-free spraying and maintain the functional properties of the coating membrane.

3.2. Morphology of casein-based films

SEM measurement of the top surfaces of films CBD_{10} and CBD_{40} was performed to study the microstructure of hybrid materials as indicated in Fig. 4a and b. In general, casein-based films showed a compact texture, and no appreciable differences were observed when the degree of casein methacrylation was varied in the synthesis. This result could be due to the fact that the use of CMC resulted in the formation of a network with improved compatibility, where the protein and acrylic portions were greatly linked. AFM analysis was also carried out to analyze the distribution of component on the film surfaces. In the AFM technique, phase images (Fig. 4c and d) allow the mechanical and viscoelastic properties of the materials to be observed. Viscous components dissipate more energy in comparison to more elastic materials, when they interact with the probe tip [34]. In the images presented here (Fig. 4c and d), acrylic regions dissipating greater energy appear darker, whereas lighter regions correspond to casein. It can be seen that casein was uniformly distributed on the film surfaces surrounding the
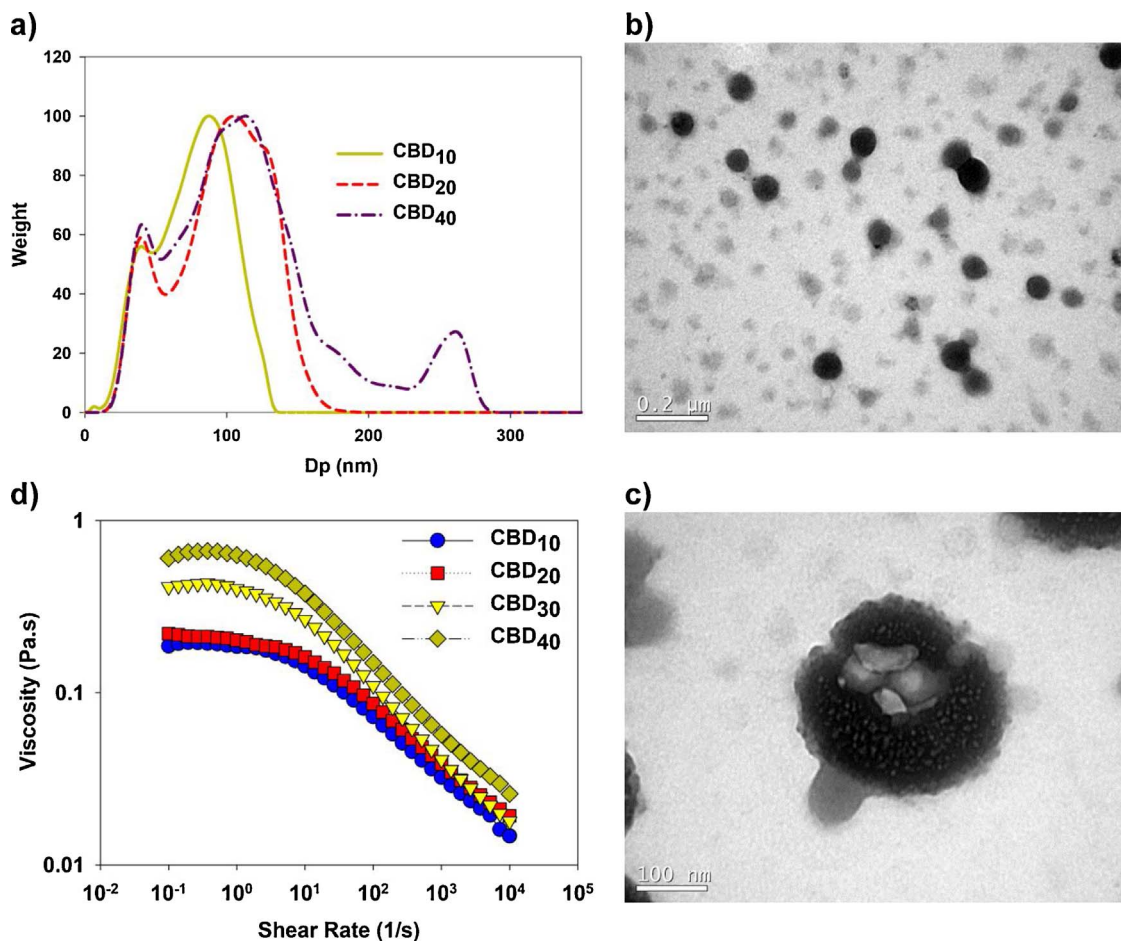
Fig. 2. Dp and fraction of macro-crosslinker consumed for protein-based dispersions.

Fig. 3. (a) Weight PSDs of casein-based dispersions; (b,c) TEM of latex particles CBD$_{40}$ and (d) viscosity profiles of dispersions.
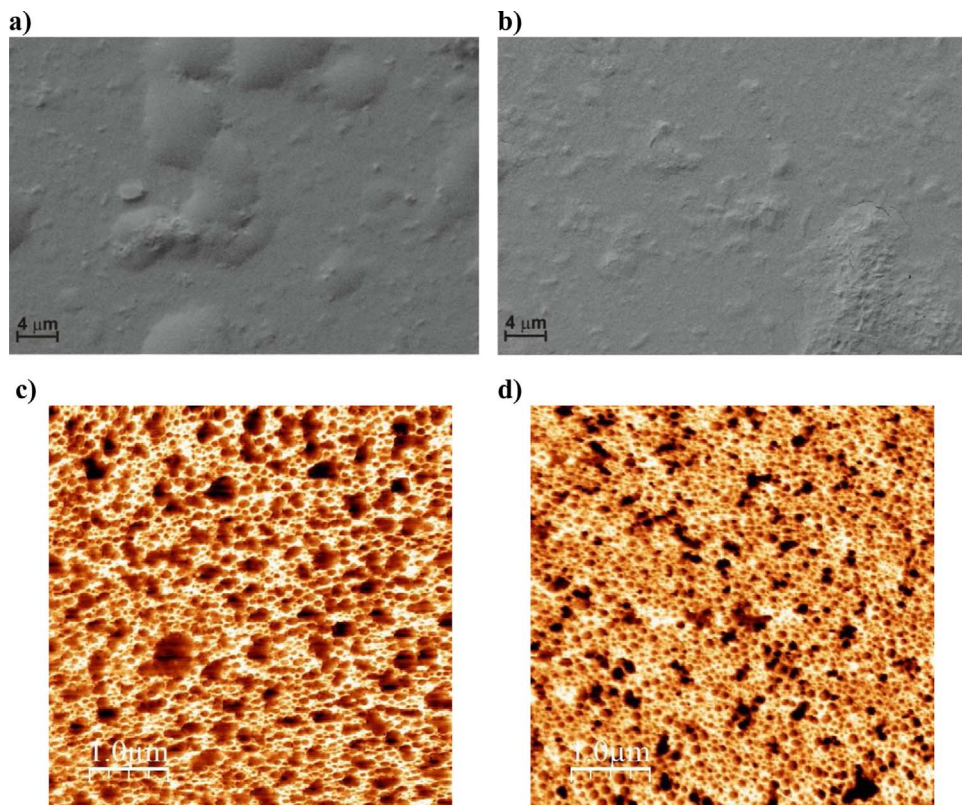

Fig. 4. SEM images (8K X) (a,b) and AFM phase images (5 × 5 μm) (c,d) of top surface of the casein-based films CBD$_{10}$ (a,c) and CBD$_{40}$ (b,d).

soft acrylic phase. This casein distribution is only achieved when both components are highly compatibilized and the molecular mobility of the protein during film formation is restricted [21]. Thus, an increase in the superficial casein concentration was observed when the number of vinyl functionalities in the protein macro-crosslinker increased from 10 (Fig. 4c) to 40 (Fig. 4d). It should also be noted that the film morphology observed in Fig. 4c and d showed that casein particles were completely coalesced despite the high glass transition temperature of this protein (above 180 °C). This result suggests that water acts as a plasticizer, favoring film formation.

3.3. Properties of casein-based materials

As previously mentioned, the poor mechanical properties of casein are the major drawbacks avoiding their widespread use in film coats. In this work, the tensile strength and elongation at break of the films prepared from CMC were taken as the key parameters to analyze the effects of the degree of casein methacrylation on the mechanical behavior of materials. The stress–strain results shown in Fig. 5a demonstrated that the degree of methacrylation did not significantly affect the tensile strength of the films, reaching in all cases a limit value around 8 MPa. On the other hand, the films exhibited decreasing elongation at break, increasing the degree of casein methacrylation. Thus, the elongation capability of the hybrid materials decreased from 672 to 387% for CBD_{10} to CBD_{40}, respectively. This behavior is attributed to an increase in the crosslinking density of the polymer bulk with the increase in the macro-crosslinker vinyl functionalities, which promotes the formation of stiff networks inside the films.

In order to confirm the increase in polymer crosslinking density, the dynamic viscoelastic behavior of the polymer films was investigated. Fig. 5b shows the storage (G′) modulus, as a function of temperature for the samples. It can be seen that G′ becomes higher with the increase in

the degree of casein methacrylation, indicating the major crosslinking density of the materials.

3.4. pH-response swelling of films

The swelling characteristics of casein-based crosslinked films were investigated at different pH. Casein is a phosphoprotein endowed with amphiphilic properties [35,36] whose IP is approximately 4.6. Therefore, it is expected that casein-based films are sensitive to pH changes.

From the results shown in Fig. 6, it can be observed that the swelling ability of the materials in the neutral (pH 7.4) and alkaline (pH 10) buffer solution was major in relation to the acid buffer solution (pH 2). At the lowest analyzed pH, a lower DS_t may be attributed to hydrogen bonds interactions between carboxylic acid (−COOH) and hydroxyl (−OH) groups from amino acid residues of casein [37]. In neutral (pH 7.4) and alkaline (pH 10) milieus, the carboxylic acid groups on the casein-based materials are mostly in an ionized state (−COO⁻), the resulting effect being more important at pH 10. In this last case, water uptake is more significant due to the electrostatic repulsion between the ionized acid groups generating swelling force [38]. Moreover, as was expected, regardless of the pH of the buffer solution, the higher the degree of methacrylation of the casein macro-crosslinker, the lower the DS_e of the films. Thus, DS_e varied from 23 to 8% at pH 2; from 120 to 70% at pH 7.4; and from 170 to 82% at pH 10, for CBD_{10} and CBD_{40}, respectively.

Also, note that the dynamic swelling curves exhibited an overshooting effect, namely, the films firstly swelled to a maximum value followed by gradual deswelling until swelling at the equilibrium. It could be observed that this effect was much more pronounced at pH 2 (Fig. 6a), followed by pH 10 (Fig. 6c) and pH 7.4 (Fig. 6b). This phenomenon is attributed to the dynamic formation of hydrogen bonding between the carboxyl and hydroxyl groups of the casein in an acidic environment [39]. Under acidic conditions, hydrogen bond interactions promote the rearrangement of the film structure into a more compact network, with lower swelling capability. This new structure has a water content higher than the value at equilibrium, and as a consequence water release and deswelling occur. Also it can be noted that the films exhibited a negligible overshooting effect at pH 7.4, probably due to the fact that COOH groups from glutamic and aspartic acid are mostly deprotonated under this condition, preventing hydrogen bonding interactions. However, as the pH is increased until 10, charged NH_3^+ from lysyl and arginyl residues becomes deprotonated and NH_2 hydrogen bonding interactions may contribute to the overshooting effect as is observed in Fig. 6c

On this point, it is worth mentioning that the overshooting effect was not related to the mass loss of the films, since this property resulted in around 12% for all samples (see Fig. S3 of the SM).

In the production of film-coating for solid dosage forms, formulations must be designed by taking into consideration that the pH of the gastrointestinal tract varies from 1.2–2.0 in the stomach to 7–8 in the intestine [40]. Therefore, casein-based crosslinked films may be used as suitable functional coatings for intestine-specific drug delivery.

3.5. Rheological studies

Due to the fact that the produced materials are intended as enteric coating for intestine-specific drug delivery applications, we performed a study of the viscoelastic properties of the films swollen in simulated physiological fluids. Therefore, the responses into the LVR of the swollen films in SGF (pH 1.2) and PBS (pH 6.8) were studied. For the different films swollen in either SGF or PBS, Fig. 7 shows the G' obtained for dynamic sweeps of angular frequency. The results show that the viscoelastic behavior of hybrid polymers swelling in PBS or SGF is as expected, where storage module G' is higher than loss module G'' in the range of frequencies analyzed (G'' is not shown in Fig. 7 for space reasons). It can be observed that the storage moduli of the films in
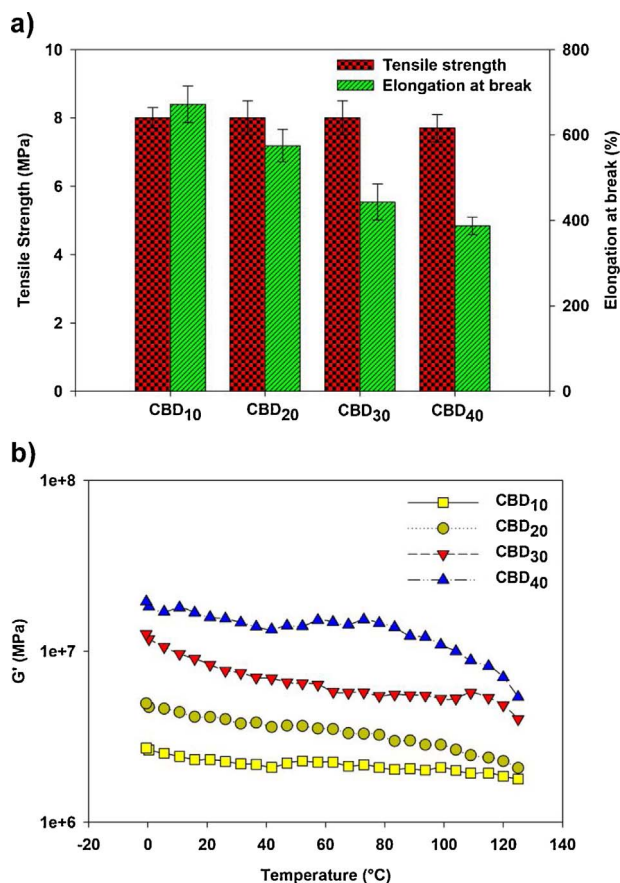

Fig. 5. Tensile test (a) and dynamic viscoelastic behavior (b) of casein-based films.
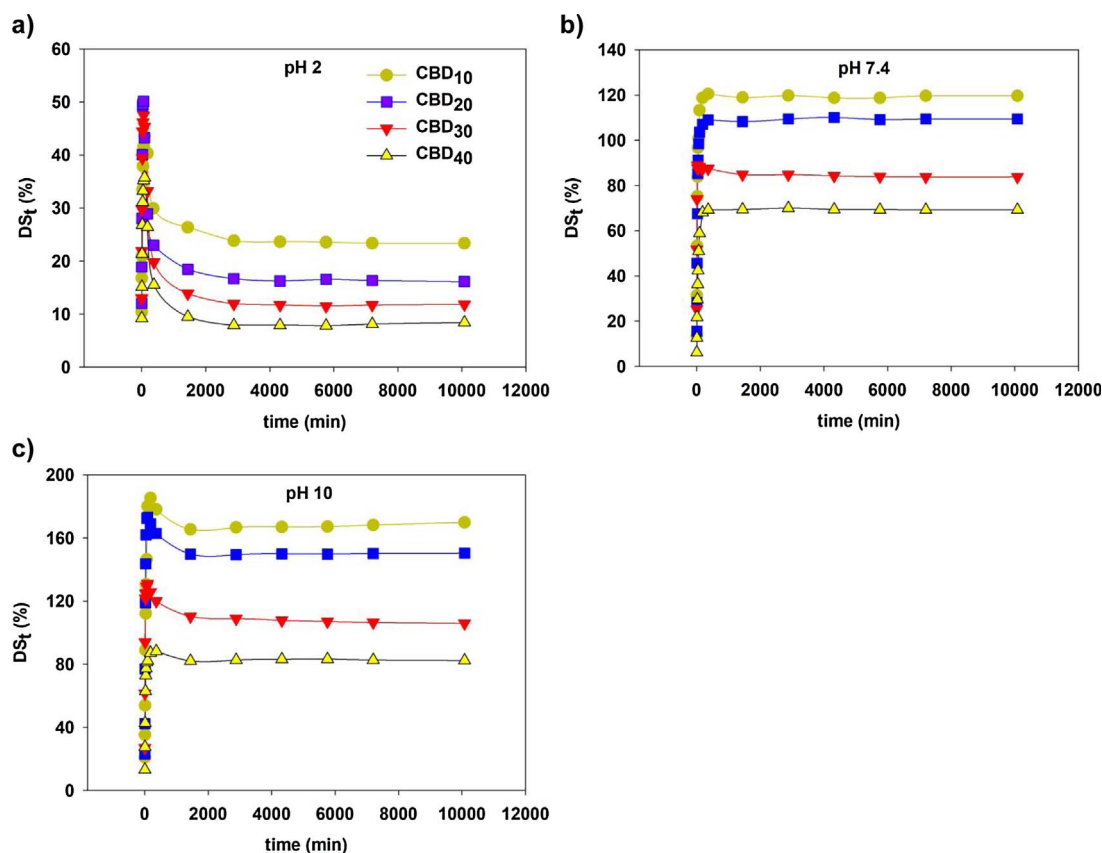
Fig. 6. Swelling kinetics of the films in buffer of pH 2 (a), 7.4 (b) and 10 (c).
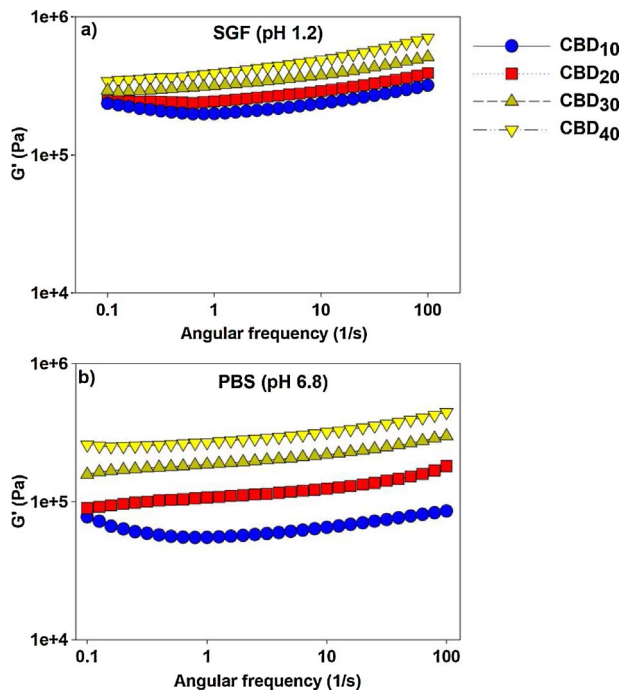

Fig. 7. Dynamic sweeps of angular frequency for films swollen in SGF (a) and PBS (b).

either PBS or SGF were slightly increased with the frequency of oscillation, which is typical of high molecular weight polymers in their rubbery region. Moreover, it can be clearly seen that the higher the degree of casein methacrylation used, the higher the G' values in both physiological milieus, which is in agreement with the results shown in Fig. 5.

It should also be noted that casein-based films presented widely different values of G' according to the pH of the buffer in which they had been previously swollen. For instance, in the case of CBD_{40}, the G' value at 100 Hz was approximately 7.0×10^5 Pa in SGF, pH 1.2 (Fig. 7a) and close to 4.4×10^5 Pa in PBS, pH 6.8 (Fig. 7b). As previously discussed, under alkaline conditions (at pH higher than casein IP) carboxylic acid groups are in an ionized state and as a consequence chains repulsion and polymer network expansion is produced. This phenomenon led to a higher fluids absorption capability and consequently the network became softer, achieving lower G' values for the swollen samples.

This result is very interesting for a potential application of the films as enteric coating in solid dosage formulations. The microstructure of these films could be reordered from a stiff matrix to a soft gel when the pH environment of the gastrointestinal tract changes from acid to alkaline. In this way, the active ingredient could be protected from the acidity of the stomach and delivered in the intestine with an optimal release profile [41].

3.6. In vitro drug release

BSA release behavior from casein-based films was studied to evaluate their potential for an oral drug delivery application. Fig. 8a shows BSA release profiles from discs containing 500 mg of that protein per gram of polymer. Using SGF as the receptor medium, a relatively slow release of BSA from CBD_{10} and CBD_{40} was observed. Thus, during the first 1 h period, the cumulative BSA release was found to be 29% for CBD_{10} and 14% for CBD_{40} films, respectively. However, in the case of PBS, the amount of BSA released increased significantly to 77% and 48% for CBD_{10} and CBD_{40}, respectively, after the same period of time. These results could be attributed to a greater swelling at pH 7.4, which is explained by the fact of COOH ionization in casein chains, which led to the extended state of the polymer network giving faster drug
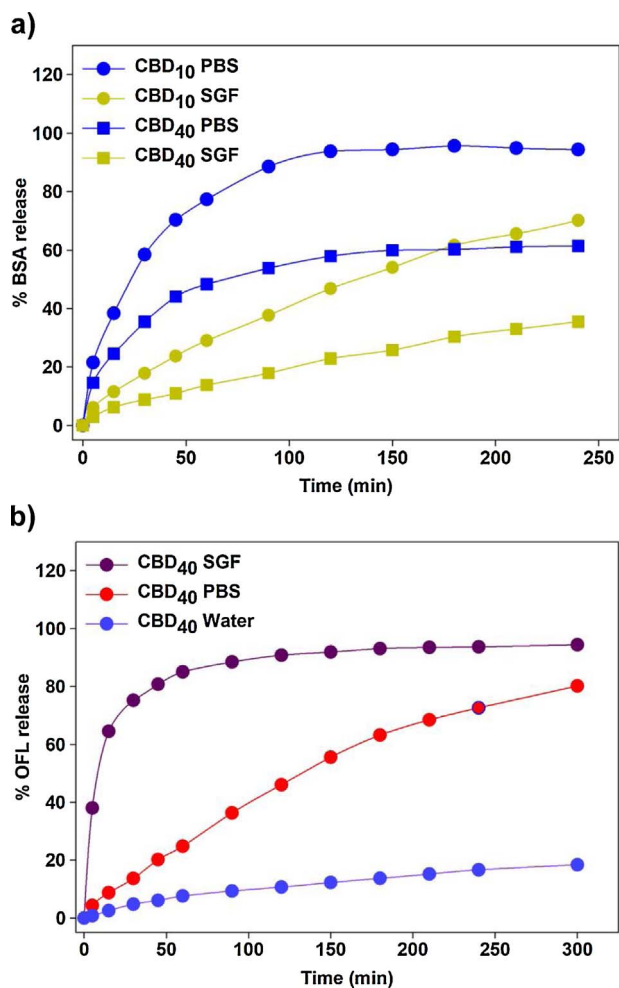
a)

b)

Fig. 8. In vitro release behavior of BSA (a) and OFL (b) from the casein-based crosslinked films at different receptor media.

Table 1
Release behavior of BSA from casein-based films.

Casein-based film	pH	n	k	R^2	Transport mechanism
CBD_{10}	1.2	0.51	0.105	0.999	Case III
	6.8	0.55	0.094	0.999	Case III
CBD_{40}	1.2	0.61	0.032	0.996	Case III
	6.8	0.63	0.106	0.998	Case III

mechanism. Then, by plotting $\ln(M_t/M_\infty)$ versus $\ln(t)$, n and k values could be obtained. According to the n value, four different mechanisms of transport could be distinguished: (i) Fickian or Case I ($n = 0.5$), in which the rate of diffusion is much less than that of relaxation during transient sorption; (ii) non-Fickian or Case II ($n = 1$), where diffusion is very rapid compared with other relaxation processes; (iii) Case III ($0.5 < n < 1$), where the transport process is anomalous, and the structural relaxation is comparable to diffusion; and (iv) pseudo-Fickian ($n < 0.5$), where the sorption curves look like Fickian curves, but with a very slow approach to final equilibrium. Table 1 shows the values of n, k and the coefficients of determination (R^2) for both analyzed films. All systems showed Case III release behaviour, with n values ranging from 0.5 to 0.65.

On the other hand, the presence of casein turns films into an ionic hydrophilic polymer with valuable properties for ionic conjugation of ionizable drugs. In order to evaluate the ability of these materials to form ionic pairs with basic drugs, OFL was used as a model. OFL is a zwitterionic molecule with antibacterial properties, which is approved for use by the oral route, in the treatment of gastrointestinal infections, among others [43]. Moreover, the presence in OFL of ionizable ternary piperazine amine group, able to form acid − base interaction with the carboxylic groups of casein, makes this drug an interesting model for release studies. Fig. 8b shows the release behavior of OFL from discs containing 300 mg of drug per gram of polymer. CBD_{40} was chosen for the analysis due to its higher crosslinking density. As can be observed, a very slow release of OFL was reached when distilled water was used as a receptor medium. The cumulative percent of total drug released after 5 h was < 20%. On the other hand, in SFG or PBS the drug release rate was significantly increased. Thus, the percent of total drug released after 5 h was 94% and 80% for SGF and PBS, respectively. Here, it is noteworthy that the saline composition of physiological fluids would trigger OFL release from casein-based films. The release results could be explained by taking into consideration that pK$_a$ of OFL piperazine amine groups is around 8.2 and hence at the final dispersion pH (about 6.5), casein and OFL have opposite charges. Then, a high proportion of the drug is present in the form of ionic pairs. Thus, if delivery occurs through the diffusion of free species, the strong ionic interaction slows down the delivery rate, as observed when water was used as the receptor milieu. Meanwhile, at pH 1.2 (SFG buffer) both casein and OFL are positively charged and ionic repulsion promotes a fast delivery. Note that although strong ionic interactions are present at pH 6.8 (PBS buffer), a substantial release rate was observed due to the fact that Na$^+$ and PO$_4^{3-}$ diffuse into the film. Thus, the exchange of cationic and zwitterionic species facilitates the release of OFL attached to casein chains.

In addition, it is worth mentioning that diffusional limitations do not seem to be present when using OFL as a model drug, probably due to its lower molecular weight (361.3 g/mol) in comparison to BSA.

4. Conclusions

Novel pH-sensitive crosslinked films were prepared exploiting the ionic properties that depend on pH, originated from casein. It was evidenced that the number of methacrylic functionalities of the casein macro-crosslinker had a great influence on the microstructure and rheological properties of the film-forming dispersions. Thus, when a

diffusion. It is also noted that in the same pH medium, the cumulative BSA release from casein-based films decreased with the increase in the macro-crosslinker functionalities used for their preparation. Furthermore, it is interesting to observe that while the BSA was almost completely delivered from the CBD_{10} film at pH 6.8, the release rate was levelled off at 60% for CBD_{40} under the same pH conditions. This result suggests that the high degree of crosslinking reached in CBD_{40} could sterically hinder the diffusion of the high molecular weight BSA (66 kDa) to the medium. At pH 6.8, wherein the film swells to a greater extent, a fast release rate was observed at the beginning of the test, probably due to the dissolution of the BSA concentrated on the film periphery. However, when this amount of BSA was completely dissolved, the protein trapped inside the film could not be delivered in the studied period of time, reaching a release plateau. Similar behavior is expected for the release in acid pH, but in this case the lower release rate due to the minor swelling ability in these conditions did not allow the drug delivery plateau to be observed in the evaluated period of time.

As a further investigation, the following semi-empirical equation [42] was used to analyze the drug release behavior of BSA from casein-based films:

$$\frac{M_t}{M_\infty} = kt^n \,(\text{for } M_t/M_\infty \leq 0.6) \tag{3}$$

where M_t and M_∞ are the concentration of BSA released at time t and equilibrium respectively, k is a constant characteristic of the system and n is the release kinetic exponent that determines the type of transport

high degree of methacrylation was used, interparticulate crosslinking could be produced, generating large particle agglomeration and increasing the dispersion viscosity. SEM and AFM analysis revealed that the films were smooth and presented an even casein distribution on their surface. Moreover, the tensile properties of the films could be tuned by varying the functionalities of the casein macro-crosslinker, reaching a good relation between strength and elongation at break. The increase in the degree of casein methacrylation led to a reduction in the elongation capability of the materials due to an increase in the crosslinking density of the polymeric network, as demonstrated by the rheological analysis. The swelling kinetics of the films at different pH showed greater swelling in the alkaline medium in comparison to that in the acidic one. This selective swelling behavior affected the viscoelastic properties of the swollen films, and higher G' values were reached in SGF rather than PBS, as determined by rheological measurements. The release behavior of BSA from the films showed pH-dependence, with faster delivery rates in the alkaline medium. The ability of casein-based films to form ionic pairs with a basic drug like OFL was also evaluated in different physiological fluids, finding that pH and saline composition could trigger drug release. Finally, the results demonstrated the high potential of these hybrid materials to be used as suitable enteric film-coating in oral drug delivery applications.

Acknowledgements

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.colsurfa.2018.01.012.

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