Casein-based hydrogels: A mini-review

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Abstract

Casein-based hydrogels are biocompatible, biodegradable, renewable, easy to obtain, inexpensive, and non-toxic. They exist in different physicochemical states, e.g. particle hydrogels, which can be divided into suspensions or emulsions and macro hydrogels that are gel colloid type. These biomaterials have drawn increasing attention in recent years due to their abilities to form networks of different tensile strengths and to encapsulate, protect and release biomolecules. This mini-review outlines the recent advances in casein-based hydrogel research and the uses of casein-based hydrogels as drug delivery systems for both hydrophobic and hydrophilic molecules. The food and biomedical potential along with possible future uses of the casein-based hydrogels are discussed throughout the document.

Keywords: casein; hydrogel; drug delivery; encapsulation; biomaterial.

1. Introduction

Hydrogels are three-dimensional networks of water-soluble polymers that possess large amounts of water trapped within their structures (Klement, Lord, & Parker, 1960). Hydrogel properties depend on polymer concentration, crosslinking levels, temperature, pH, aging, and salt concentrations (Bae & Kurisawa, 2016; Ahmed, 2015). Hydrogels’ porous structures make them strong candidates for the protection and transport of bioactive compounds (Rossi, Castiglione, Ferro, Moioli, Mele, & Masi, 2016; Caló & Khutoryanskiy, 2015). Hydrogels can be formulated by using a wide range of polymers, including those of food origin. The benefits of hydrogels made from food-grade biopolymers include safety, low cost, and commercial availability (Ali & Ahmed, 2018; Le, Rioux, & Turgeon, 2017; Sun, Lv, Cao, Liu, Zhang, & Lu, 2015). One possibility for
Creating food-based hydrogels is the use of caseins, alone or in combination with other food-grade polymers.

Cow’s milk protein concentration is, on average, 30 - 35 g proteins per liter. Approximately 80 % of those proteins are caseins (Huppertz, 2013). Caseins are phosphorylated proteins that have a net negative charge in milk pH. They are proline-rich proteins, resistant to heat denaturation, with limited secondary and tertiary structures (De Kruif, Huppertz, Urban, & Petukhov, 2012). There are four main types of caseins in milk, α-s\textsubscript{1}, α-s\textsubscript{2}, β, and κ-casein with molar ratios of 11:3:10:4, respectively. There are several genetic variants of these proteins (Huppertz, 2013). Caseins are classified as rheomorphic proteins once they assume different conformations according to their physicochemical environment (Holt, Carver, Ecroyd, &Thorn, 2013). Due to the high levels of phosphorylated residues in casein molecules, the molecules tend to self-assemble forming casein micelles (CMs) in aqueous solutions supersaturated with calcium phosphate, as it the case for cow’s milk (Walstra, Wouters, & Geurts, 2006).

CMs are supramolecular aggregates formed by α-s\textsubscript{1}, α-s\textsubscript{2}, β and κ-caseins. These are held together through hydrophobic and electrostatic interactions and nanoclusters of calcium phosphate (De Kruif, 2014). The average size of CMs milk is 200 nm, but this size can vary considerably depending on milk origin (Dalgleish, 2011). CMs size is limited by the surface presence of the κ-casein. In addition to limiting CMs’ size, the κ-casein is primarily responsible for maintaining CMs in suspensions via electrostatic repulsion and steric impediments between the CMs. The main biological function of CMs is to deliver calcium to neonates and prevent mineral sedimentation in the mammary gland ducts (Huppertz, 2013). In this sense, CMs can be described as naturally occurring hydrogels, which act as an efficient encapsulation device for mineral transportation.

It is also possible to obtain caseins other than CMs in different forms, e.g. sodium caseinate (CasNa). Briefly, CasNa is obtained through CMs destabilization, which is
induced by precipitation at an acid pH, followed by the resuspension of the precipitated in a sodium hydroxide solution. The resuspended caseins are then spray dried, and the resulting powder is referred as CasNa (Walstra, Wouters, & Geurts, 2006). When CasNa is rehydrated, the organization state of caseins is dictated by the physicochemical conditions of the media.

Different types of casein hydrogels have been employed for the delivery and controlled release of a wide variety of biomolecules (Głąb & Boratyński, 2017; Ranadheera, Liyanaarachchi, Chandrapala, Dissanayake, & Vasiljevic, 2016; Spizzirri, Cirillo, Curcio, Spataro, Picci, & Lemma, 2015). These include: dispersions of particle hydrogels and macro hydrogels. The former may be divided into emulsions and suspensions (Kwan & Davidov-Pardo, 2018; Turovsky, Portnaya, Kesselman, Ionita-Abutbul, Dan, & Danino, 2015; Elzoghby, Helmy, Samy, & Elgindy, 2013 a; Semo, Kesselman, Danino, & Livney, 2007). The latter, can be physicochemically classified as gel (Xu, Fan, Duan, & Gao, 2018; Yin, Su, Qi, & He, 2011). For the sake of precision, the term ‘casein hydrogels’ is used in this study to refer to colloidal systems in which caseins are the only polymer source. When other biopolymers are forming the hydrogels, the resulting systems are referred to as casein-based hydrogels. Therefore, this mini-review presents the recent advances in casein and casein-based hydrogels, along with their use as drug delivery systems for hydrophobic and hydrophilic bioactive molecules.

The studies describing casein and casein-based hydrogels systems are summarized in Table 1. The following text was separated into three sections which correspond to the general aspects of casein and casein-based hydrogels, main types of casein particle hydrogels, and macro hydrogels.

2. General Aspects
It is essential to evaluate the cumulative release of an encapsulated bioactive when developing a hydrogel system. A favorable interaction between the drug and the delivery matrix used must be ensured; otherwise encapsulation will not occur. However, a release of the bioactive compound from the carrier matrix to the external medium must also occur (Tavares, Croguennec, Carvalho, & Bouhallab, 2014). For this reason, the drug’s release profile for a specific physicochemical environment must be studied. The medium conditions for in vitro experiments must be established to be as similar as possible to the actual conditions found in the system application. If the system is designed for oral delivery, it is important to study release conditions that replicate to conditions found in the stomach and intestine, i.e. pH and ionic strength that are similar to these environments.

Hydrogel drug release is primarily explained via three mechanisms: simple drug diffusion, hydrogel swelling/shrinking, and chemically-controlled release (Lin & Metters, 2006). Generally speaking, diffusion occurs according to Fick’s Law of diffusion. It is the most common mechanism found in a hydrogel drug release system (Lin & Metters, 2006). The diffusion rate is proportional to the concentration gradient (Walstra, 2003), thus, mass transport from the hydrogel matrix to its surroundings occurs until equilibrium is established. The diffusion rates of the encapsulated drug through the hydrogel matrix depends on pore size, polymer motion and polymer/bioactive interactions (Amsden, 1998). In general, pore size and diffusion rates of bioactive molecules are directly proportional, i.e. a higher pore size leads to greater diffusion rates (Peppas, 2019). Charged groups within the hydrogel matrix that interact with the bioactive ingredient may also retard molecular diffusion (Tan, Ebrahimi, & Langrish, 2019).

A hydrogel’s water absorption capacity allows it to swell. Swelling increases hydrogel pore size, which in turn facilitates the release of the encapsulated drug (Lin & Metters, 2006). Changes in pH, temperature and ionic strength can promote drug release or water absorption. These features can also be used in a drug delivery system. De Kruif
et al. (2015) carried out experiments to study the swelling/shrinking behavior of casein systems by measuring their water holding capacity (WHC). The studied systems included native CMs, sodium caseinate, renneted casein and CMs cross-linked with transglutaminase (Tgase), which is a transferase that catalyzes the formation of covalent bonds between lysine and glutamine residues (Huppertz & Kruif, 2008). The systems were subjected to different physicochemical conditions, including temperature variations, different salt types, varying salt concentrations, alcohol presence and urea presence. The study results showed that the WHC of casein-based hydrogels can be defined as the water entrapped in the hydrogel matrix while it is in a state equilibrium with the medium. The authors demonstrated that a hydrogel’s WHC depends primarily on crosslink density, solvent quality, and temperature.

A chemically-controlled release mechanism plays an important role in polymers. During chemically-controlled release, a hydrogel matrix is usually cleaved via enzymatic hydrolysis (Lin & Metters, 2006). Hydrogel degradation exposes the encapsulated molecule, and the release rate increases (Tavares et al., 2014). In the case of casein hydrogels, the enzymatic hydrolysis effect must be taken into consideration when a drug is administered orally and proteolytic enzymes hydrolyze the hydrogel (Cohen et al., 2017). Hydrogels may degrade also without enzymatic reactions. Hydrogel stability can also interfere with the bioactive release rate (Yin et al., 2011). Polymer/polymer interactions can be affected and weakened by the surrounding medium. The protein network degrades in response to ionic strength, temperature and pH conditions. For casein hydrogels, a pH change is the main mechanism applied to promote hydrogel destabilization. An important change in protein charge can occur when pH approaches or moves away from the isoelectric point. Casein hydrogels used in oral drug delivery undergo at least 3 abrupt pH changes: mouth pH \( \approx 7 \), stomach pH \( \approx 3 \) and intestine pH \( \approx 7 \) (Minekus et al., 2014). Therefore, an understanding of hydrogel behavior in these situations is essential to
customize the delivery system. A hydrogel stability test measures how long and at what rate the system can maintain or loosen its own structure. A negative correlation exists between hydrogel stability and drug release rate (Yin et al., 2011). When the polymer chains leave the main matrix, the overall network diminishes. This in turn facilitates drug diffusion.

As the network is the main component of the system, additional polymers can influence the hydrogel’s rheology, development time, and release rates (Xu et al., 2018; Wei et al., 2016). Interaction between caseins and additional polymers may improve the hydrogels’ mechanical properties, as well as create a stimuli -sensitive system based on changes in polymer interactions. These occur when certain parameters such as temperature and pH are altered (Zhang, Zhang, Tong, Decker, & McClements, 2015; Zhang, Zhang, Decker, & McClements, 2015). Interaction between polymers within the hydrogel matrix can be divided into weak interactions or strong interactions (Maitra & Shukla, 2014). In the case of strong interactions, covalent bonds are formed within the hydrogel matrix. For weak interactions, the polymers interact by hydrophobic, hydrogen-bonding, ionic interactions and physical entanglement of chains. For CMs crosslinking, intramicellar covalent bonds are formed within or between casein chains (Ercili-Cura et al., 2013). After crosslinking, the CMs structures are unable to dissociate in the given physicochemical environment (Huppertz & De Kruijf, 2008; Silva, Saint-Jalmes, Carvalho, & Gaucheronet, 2014). Casein hydrogels require the presence of a crosslink agent to promote a crosslink reaction. Casein crosslink agents that have been studied in the past include glutaraldehyde (Glu) (Barbosa et al., 2014; Migneault, Dartiguenave, Bertrand, & Waldron, 2004), genipin (GP) (Casanova et al., 2017; Silva et al., 2014), Tgase (Silva et al., 2018; Raak, Rohm, & Jaros, 2017; Schorsch, Carrie, & Norton, 2000), tyrosinase (Xu, Teng, & Wang, 2016) and hyaluronic acid (Li, Fu, & Zhang, 2014). Each of these crosslink
agents demonstrates advantages and disadvantages that must be evaluated when designing a hydrogel delivery system.

3.1. Particle hydrogels

Particle hydrogels are hydrogel systems with an average mean size in the micro and nano range. Bulk systems made up of these particle hydrogels may be classified as suspensions or emulsions (Shaw, 1992). In both cases, the continuous phase is liquid, generally water. When the dispersed phase is formed by a particle hydrogel encapsulating a solid bioactive compound, it is defined as a suspension. Otherwise, if the particle hydrogel is designed to encapsulate a liquid, the system is defined as an emulsion.

Silva et al. (2014) used different concentrations of GP to crosslink CMs. This crosslinking agent forms a monomeric adduct, and then it crosslinks the protein units with the participation of lysyl and arginyl residues in the reaction. The casein hydrogels were produced using 5 mM, 10 mM and 20 mM in a casein dispersion of 2.5 %. Dynamic light scattering measurements (DLS) showed a decrease in the hydrodynamic diameter (Dh) of CMs as a function of GP concentration. In the control sample (without GP) Dh was 179 nm, whereas the values for crosslinked CMs with 20 mM GP was 160 nm. The dispersion viscosities also decreased when higher GP concentrations were tested, assuming values from 1.655 mPa.s⁻¹ (control) to 1.441 mPa.s⁻¹ (20 mM GP). Those observations suggested GP crosslink process occurs internally, since no aggregates were detected by DLS, viscosity analysis and scanning electron microscopy. The presence of GP, even in the lowest concentration used in this study, was able to prevent CMs dissociation via the addition of sodium citrate and urea. The presence of covalent bonds formed between lysine and arginine residues balanced the influence of sodium citrate and urea over CMs. The increase in CMs stability promoted by GP can favor the use of this hydrogel as a drug delivery system. The encapsulation potential observed in the system was tested using
alfuzosin hydrochloride, a water-soluble drug prescribed to treat benign prostatic hypertrophy (Elzoghby, Samy, & Elgindy, 2013b). In the study, the authors developed a hydrogel set by testing protein/drug ratio, protein/GP ratio and crosslink reaction time combinations. During the study, casein hydrogels were formulated by dispersion of casein powder in 0.1 M sodium hydroxide solution. The drug was loaded by mixing the suspension for 2 h. GP was added to promote crosslink reaction. The selected GP concentrations set this study apart from previous research. The authors used 2.5, 10 and 40 % w.w\(^{-1}\) as these values were higher than values used in previous studies on using GP as a crosslink agent for caseins. Song et al. (2009) shown that drug release behavior for the casein micro hydrogels varied according to GP concentrations when exposed to phosphate-buffered solutions at pH 7.4. After 24 h of continuous drug release, the sample containing 40 % w.w\(^{-1}\) of GP was found to have released approximately 68 % of the initial drug amount whereas a 2.5 % w.w\(^{-1}\) of GP showed a release of about 88 %. The crosslinking reaction time was also shown to interfere with the release properties of the hydrogel particles. After 5 h of crosslinking reaction the samples were found to have released approximately 70 % of the loaded drug, 20 % less than the sample in which a cross-linked reaction took place for just 1 h. In summary, a longer crosslinking reaction time was shown to lead to slower drug diffusion regardless of hydrogel system size. At the same time, high GP concentrations were also shown to lead to a slower drug release rate. According to the authors, this behavior was primarily due to the smaller pore size of the hydrogel matrix caused by a denser network structure.

Use of tyrosinase as crosslink agent has grown in the last years. In combination with certain phenol groups, tyrosinase can achieve similar crosslink results when compared to Glu and GP. Tyrosinase is a polyphenol oxidase enzyme naturally present in fruits. Its potential use as crosslink agent for casein was studied by Xu, Teng, and Wang (2016) (Xu, Teng, & Wang, 2016). The authors employed pyrocatechol, a polyphenol
present in certain fruits (Monforte et al., 2014) and chlorogenic acid hemihydrate, a polyphenol found in coffee and certain herbs (Mikami & Yamazawa, 2015). The casein hydrogel particles formulated with tyrosinase – pyrocatechol and tyrosinase – chlorogenic acid hemihydrate showed greater crosslink efficiency than the crosslink reaction with Glu. The reaction between the enzyme and the phenol compounds formed quinone groups that reacted with the amide, amine and carboxyl groups present in casein, promoting the crosslink reactions. Tyrosinase-phenol crosslinks were shown to have low toxicity, and the extension of crosslink sites was shown to be directly proportional to the concentration of phenol molecules. However, there are few studies describing the use of tyrosinase as a crosslinking agent for the development of casein hydrogels. More studies are needed on the subject. Although the crosslink efficiency of tyrosinase is similar to that of well-established crosslink agents such as Glu and GP, it requires an additional component, i.e. polyphenol groups, for the reaction to occur.

Besides their ability to deliver molecules, casein systems can also protect an encapsulated component against degradation. A simple emulsion formed only solely of CasNa as an emulsifying agent gave lipid molecules an increased resistance to oxidation (Matalanis, Decker, & McClements, 2012). Additionally, use of casein hydrogels in these systems diminishes lipid release ratios, which may be desirable certain applications such as delivery of lipophilic bioactives and increase of lipid stability. Some systems used to encapsulate lipids have been developed by combining a CasNa crosslink with Tgase and another polymer. Matalanis, Decker, and McClements (2012) developed a CasNa hydrogel that was combined with high methoxyl pectin (HMP). The authors compared 3 different systems using: tween 20 as an emulsifier, CasNa as emulsifier and CasNa/HMP hydrogels. The authors highlighted how casein-based hydrogels were able to limit lipid degradation during 17 days of storage. The lipid degradation rate was approximately 3 times slower in the samples containing casein-based hydrogel than in the rate for the
The authors suggest that the oxidation mechanisms were distinct. For the CasNa oil emulsion, it has been suggested that the casein present in the aqueous phase could limit lipid degradation because the protein reacts with transition metals and prevents contact between them and the lipid droplets. In the CasNa hydrogel, the lipid droplets were more similar to an extensive protein network where the proteins’ ability to scavenge free radicals would be crucial in preventing oxidation. Both the CasNa hydrogel and the CasNa emulsion experiments presented interesting results for limiting lipid oxidation, although the release properties for the two systems are distinct. The CasNa hydrogels were suggested to slow lipid release rates, once more complex networks were established.

A similar system was developed to encapsulate fish oil by Salcedo-Sandoval et al. (2015). The fish oil was encapsulated in a shell containing crosslinked CasNa. After that, the system was given a pectin coating. The system was used to enrich pork meat with fish oil. The experiment tested situations where the fish oil was introduced as such, using a CasNa emulsion without crosslinking, and using crosslinked CasNa plus pectin (casein-based hydrogel). The casein-based hydrogel particles were shown to be more effective in lowering oxidation rates than the other emulsion systems during 19 days of storage. Once the casein-based hydrogel was injected into the pork meat, the levels of thiobarbituric acid reactive substance (TBARS) were lower than those found in the CasNa emulsion (approximately 59 % less) and also lower than those found when the fish oil was directly added to the meat (80 % less). These results demonstrated the benefits of using a crosslinked casein-based hydrogel to prevent lipid oxidation and facilitate the addition of fish oil to meat products and develop functional foods.

Composite hydrogel particles formulated using CasNa and low methoxyl amidated pectin (LMP) have been used to encapsulate, protect and release fish oil under simulated small intestine conditions (Zhang, Decker, & McClements, 2014). LMP is an anionic
biopolymer which interacts with CasNa with electrostatic forces below pH 4.6. CasNa was essential to formulate the desired hydrogel using the following method: a thin layer of CasNa was applied around the lipid droplets followed by a second layer of LMP. The LMP then interacted with the caseins via the electrostatic interaction that occurred when pH was modified. The protein-carbohydrate interaction enabled the formulation to efficiently encapsulate and limit fish oil degradation during storage. In small intestine conditions, the fish oil was released from the hydrogel network through the weakening of electrostatic forces and through protease action in the hydrogel protein phase.

Zhang et al. (2015) (Zhang et al., 2015a) have studied the combination of CasNa and alginate as delivery systems for hydrophobic molecules. In their research, they focused on alginate, a negatively charged polysaccharide that interacts with caseins when they are below their isoelectric point. The authors showed that the CasNa and alginate ratios are crucial to end-result hydrogel features. The interaction strength varies when active sites increase or decrease. The study evaluated 3:1 and 1:4 ratios of casein to alginate. When casein was at a higher concentration than alginate (3:1) the diameter of the particles was close to 700 μm. This was 175 times higher than the diameter recorded when the alginate concentration was higher than casein (1:4). Carbohydrate presence also affected protein aggregation. This in turn led to smaller particle formation when higher amounts of alginate were present. Altering pH caused changes in charge distribution of both polymers. Stronger interactions occurred when polymer charges were opposite (low pH). Even though the experiments showed that encapsulating corn oil in a casein-alginate hydrogel was possible, the hydrogel only remained stable in a pH range of 4.0 to 5.0. This narrow pH range ultimately reduces the hydrogel’s applicability. However, when used in food systems, CasNa-alginate hydrogels may protect lipid droplets during storage so that the fat is released only when it comes in contact with saliva.
Zhang et al. (2015) (Zhang et al., 2015 b) have also formulated hydrogel particles using CasNa and gelatin. Gelatin’s low melting point facilitated lipid release under simulated oral delivery conditions. The CasNa-gelatin hydrogel particles enabled lipid delivery at temperatures of approximately 35 °C, which is close to human body temperature. The CasNa emulsifying properties were responsible for homogeneous lipid droplet size and their net charge promoted complexation with gelatin. Although the hydrophobic molecules were released into an oral environment, dissociation occurred at a low complexation temperature, a factor which could diminish the applicability of this delivery system. According to the authors, CasNa-gelatin hydrogels may be used as an oral delivery option in tropical countries, where temperatures can reach 35 °C, more readily than in temperate regions.

3.2. Macro hydrogels

Macro hydrogels are produced when a continuous polymeric network is formed using physical and/or chemical crosslinking, or when polymer chains become mechanically entangled (Shaw, 1992). The network is able to retain a solvent (dispersed phase). Solvent entrapment lends the system a solid-like appearance. In rheology, gels can be classified as soft or elastic solids (Walstra, Wouters, & Geurts, 2006), depending on whether the system has an elastic modulus that remains higher than the viscous modulus.

Casein hydrogels, created using GP as crosslink agent were studied regarding their rheological properties (Song, Zhang, & Yang Yan, 2009). A suspension of 8 % w.w\(^{-1}\) sodium caseinate was reacted with 2.5, 5 and 10 mM GP. Oscillatory rheology measurements were carried out and gelation time was determined as the cross-over between the G’ and G” curves. The control treatment (casein dispersion without GP) did not present a cross-over between the two curves, as viscous modulus (G”) was greater than storage modulus (G’) throughout the experiment. The gelation time for the sample
containing 10 mM of GP was 6.47 times shorter than that of the sample with 2.5 mM GP. These results suggest a denser network formation when GP amounts are increased, with higher GP levels leading to stronger hydrogels. Casein hydrogels crosslinked with GP also demonstrated temperature dependence. At 35 °C the cross-over between G' and G'' occurred in 44.7 min, whereas at 50 °C it occurred in 27.6 min for the same GP concentration (5 mM). Casein hydrogel properties can therefore be modified by altering temperature and GP concentrations. The hydrogels created were then used to encapsulate and release bovine serum albumin (BSA) under pH 1.2 and 7.4. The release of BSA was slower at acid pH levels (34.7 % in 5 h) than at alkaline pH levels (60.1 % in 5 h) for the same GP concentrations. The release of encapsulated molecules is influenced by the behavior of the carrier matrix in a specific medium.

As previously discussed, hydrogel swelling can be a useful tool to promote drug release. A faster release of BSA from the hydrogel was observed when it was immersed in pH 7.4 (a level is close intestine pH). The higher release rate facilitates drug diffusion through the hydrogel matrix. This phenomenon occurs because most carboxyl groups are unprotonated at pH levels over the isoelectric point of caseins. This in turn causes a repulsive electrostatic force increase between the protein chains. The electrostatic repulsion increase promotes an increase in network pore size, which facilitates BSA diffusion.

Microbial Tgase, an enzymatic crosslink agent has also been used to induce the formation of a casein hydrogel (Song, Zhang, Shi, & Li, 2010). Dynamic rheological measurements were used to understand the modification caused in the hydrogel structure by changing Tgase concentrations and crosslink reaction temperature. The ability to carry out a controlled delivery of vitamin B12 in a pH environment similar to intestine pH (PBS solution, pH 7.4) was evaluated and correlated for the hydrogel system structure. Vitamin B12 is a water-soluble vitamin present in a wide range of animal food products such as
milk, eggs, and meat. Tgase concentrations were shown to influence the hydrogels' gelation times. Hydrogels treated with 0.05 % Tgase had gelation times that were 1.81 times slower than those treated with 0.2 % of the enzyme. Tgase crosslinking reactions were also found to be temperature-dependent. At 30 °C, gelation time was approximately 115 min, whereas at 50 °C, gelation time was just 20 min. It can be observed that the 20 °C temperature increase induced a gelation rate, that was 5 times faster than the original rate. This is because 50 °C is the optimal temperature for Tgase activity. At 50 °C the rate of covalent bounds formation reaches its maximum (Schorsch et al., 2000); thus, the hydrogel structure is formed in less time. Tgase concentrations also interfered with the vitamin B12 release behavior. At Tgase concentrations of 0.05 %, 60 % of the vitamin B12 was released in 2 h, compared to just 25 % for a 0.2 % Tgase concentration. This difference may be due to the extent of crosslink sites presented in the hydrogels. Higher Tgase concentrations led to more crosslinks, which in turn contributed to a more compact structure that ultimately made drug mobility more difficult.

Casein-based hydrogels can also be created using Tgase and as konjac glucomannan (KG), a non-toxic polysaccharide as an additional polymer (Yin et al., 2011). A dispersion of 10 % w.w⁻¹ caseins was crosslinked with 0.4 % w.w⁻¹ Tgase in the presence of KG in concentrations of 0.5 and 1 % w.w⁻¹. The polysaccharide was shown to alter hydrogel formation, stability, swelling and drug release properties. When higher amounts of KG were present, shorter gelation times were observed during hydrogel formation. The authors attribute this phenomenon to an increase in continuous phase viscosity caused by the carbohydrate. Hydrogel stability was evaluated by immersing samples in a phosphate buffer solution (PBS) at pH 7.4, followed by protein quantification of the supernatants. The samples containing the highest KG levels showed higher stability results. Casein hydrogels with 1 % w.w⁻¹ of KG reached 56.6 % degradation levels after 75 h of testing, whereas hydrogels without KG showed 49.8 % degradation in 7 h. These
Results illustrate the benefits of using multiple substances in hydrogel formulations. The hydrogels formulated were then used to encapsulate and control the release of docetaxel, a hydrophobic molecule with anti-neoplastic properties (Hurtubise & Momparler, 2004). The hydrogels with high KG levels were found to have a slower release rate compared to the samples made up solely of caseins and Tgase. The casein-KG hydrogels demonstrated a release rate of 77.3 % in 30 h, whereas the release rate for the hydrogel without KG was 73.3 % in 8 h at pH 7.4.

A casein-based hydrogel made with yak milk caseins and hyaluronic acid has been formulated by Li, Fu, and Zhang (2014) (Li, Fu, & Zhang, 2014). Hyaluronic acid is a natural polymer present in biological fluids and tissues. It has viscoelastic properties that make it useful in many biomedical applications (Kogan, Šoltés, Stern, & Gemeiner, 2007). Hyaluronic acid was treated with sodium periodate to promote the formation of free aldehyde groups responsible for reacting with caseins. Covalent bonds were formed between the hyaluronic acid free aldehyde groups and the caseins amino groups. Free aldehyde groups were shown to play an essential role in gelation, thus no gelation occurred when untreated hyaluronic acid was used. The sample prepared with 3 % w.w\(^{-1}\) of oxidized hyaluronic acid presenting about 5 % of free aldehyde groups achieved sol-gel transition in 11.1 min. This study presented an alternative method for hydrogel formation.

In order for the crosslink reaction to occur, free aldehyde groups must be present in the hyaluronic acid, and crosslinking intensity was proportional to free aldehyde group quantities. The crosslinking mechanism did not require the use of additional chemical agents to promote hydrogel formation. The hyaluronic acid acted as both copolymer and crosslinker. The swelling ratio was found to be inversely proportional to the concentration of free aldehyde groups. This fact is also attributed to the more compact structure of the hydrogels that had more free aldehyde groups, which was confirmed using scanning electron microscopy (SEM). These casein-based hydrogels did not present any
cytotoxicity, making them potential candidates for use as a drug delivery system for hydrophilic compounds.

The hydrogels described above demonstrate similar mechanical resistance capacities. By contrast, a highly stretchable and notch-insensitive hydrogel has been developed using CMs and polyacrylamide, a polymer made up of photo-crosslinked acrylamide monomers (Ma et al., 2016). Acidification of the medium was necessary to promote CMs proximation, which occurred due to charge neutralization. This led to the weakening of electrostatic repulsion between CMs. The formulated hydrogels were found to be stretchable to over 35 times their original length. Hydrogel notch-sensitivity was determined for the hydrogels by placing notches of varied sizes over each, then stretching them to test their ability to return to their original sizes. The notches were observed returning to the original sizes even after the hydrogel had been stretched 28 times longer than its original length. The authors also compressed the samples. The hydrogels formulated using CMs crosslinked with polyacrylamide returned to their original shapes after compression whereas hydrogels formulated using only CMs and only polyacrylamide were more brittle and did not return to their original shapes. CMs friction and deformation were given as possible explanations. The authors also suggest that the CMs may absorb and spread the stress applied to the polyacrylamide network that maintains the hydrogel structure.

4. Conclusions and future directions

As this mini-review indicates, caseins have inherent properties that make them an excellent candidate for smart hydrogels that release biomolecules in various environments. These casein-based hydrogels have a wide range of possible applications in the food, pharmaceutical and biomaterial industries. Moreover, casein-based hydrogels’ water absorption and swelling capacities allow the entrapment of selected molecules and a
subsequent controlled release. A growing number of studies focusing on casein-based hydrogel structure continue to appear in scientific journals. This trend demonstrates the vast opportunities this field of study offers. More comprehensive research is required to further the understanding of casein-based hydrogel structural properties and determine potential applications for these soft materials on an industrial scale.

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6. Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

7. References


Table 1 – Casein and casein-based particles and macro hydrogels as carriers of bioactive compounds.

<table>
<thead>
<tr>
<th>Polymer(s)</th>
<th>Colloid type</th>
<th>Encapsulated molecule</th>
<th>Crosslink agent</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caseinate</td>
<td>gel</td>
<td>BSA</td>
<td>GP</td>
<td>Song et al., 2009</td>
</tr>
<tr>
<td>Caseinate</td>
<td>gel</td>
<td>Vitamin B12</td>
<td>Tgase</td>
<td>Song et al., 2010</td>
</tr>
<tr>
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<td>gel</td>
<td>Docetaxel</td>
<td>Tgase</td>
<td>Yin et al., 2011</td>
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<tr>
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<td>Fish oil</td>
<td>Tgase</td>
<td>Matalanis et al., 2012</td>
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<tr>
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<td>suspension</td>
<td>Alfuzosin</td>
<td>GP</td>
<td>Elzoghby et al., 2013</td>
</tr>
<tr>
<td>Sodium caseinate + Low methoxyl amidated pectin</td>
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<td>Fish oil</td>
<td>Tgase</td>
<td>Zhang et al., 2014</td>
</tr>
<tr>
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<td>Salicylic acid</td>
<td>–</td>
<td>Li et al., 2014</td>
</tr>
<tr>
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<td>Corn oil</td>
<td>–</td>
<td>Zhang et al., 2015</td>
</tr>
<tr>
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<td>emulsion</td>
<td>Corn oil</td>
<td>–</td>
<td>Zhang et al., 2015b</td>
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<td>Tyrosinase + phenols</td>
<td>Xu et al., 2016</td>
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<tr>
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<td>Polyacrylamide</td>
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<td>Tgase</td>
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<td>Ding et al., 2016</td>
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<td>suspension</td>
<td>Nile red</td>
<td>Glyceraldehyde</td>
<td>Picchio et al., 2018</td>
</tr>
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</table>
Declaration of interests

☑ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:
Casein-based hydrogels are suitable for use in biomedical applications.

Caseins hydrogels in different colloidal states can deliver bioactive compounds.

Interactions with other polymers can widen casein hydrogels applicability.