Hydrogel characteristics resulting in increased cargo stability: a mini-review

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Abstract

Over the years, hydrogels have gained significant interest within the biomedical field. The high tunability of physical and chemical properties allows them to be used for very versatile applications, such as their excellent drug delivery properties. These drug delivery properties are accompanied by increased chemical and physical stability of load while encapsulated in the hydrogel. Hydrogels have been used to carry a wide array of cargo, ranging from small molecules to biotherapeutics such as proteins, peptides or nucleic acids and have shown different characteristics that contribute to increased stability. This mini-review summarizes these characteristics that can be introduced and tuned in the hydrogel network to achieve a stability increase for the carried products.

Keywords :

Hydrogel, Cargo, Stability, Degradation, Protection

1. Introduction

Hydrogels are three-dimensional hydrophilic polymer systems that can take up a minimum of 10-20% [1,2] and up to over 99% of water[3], yet do not dissolve. The high degree of versatility in chemical and physical properties, in combination with unique characteristics that can contribute to creating a biocompatible environment, allows hydrogels to be a useful tool for many different biomedical applications.[4,5] Hydrogels have been used for tissue engineering[6], contact lenses[7], tissue regeneration[8] and stem cell expansion.[910] Additionally, hydrogels have been used as delivery systems for small molecules, proteins, nucleotides and advanced medicinal products.[10,11,12] The high degree of tunability in the chemical and physical properties of hydrogels allows for the design of systems that can release load based on specific triggers (i.e., pH, temperature, radiation). Hydrogels serve multiple functions in drug delivery including targeted treatment[13,14], sustained release [15]and/or reducion of cargo degradation.[16]

Protein and peptide drugs often have short half-lives [11], but certain small molecule drugs have also shown to be cleared rapidly. [17] Encapsulation in hydrogels has been related to increased pH- and thermo-stability of biological products.[18] Additionally, protection from the immune system and delayed clearance while encapsulated have also been described as factors in half-life improvements observed through reduced interaction of humoral and cellular immune response with biological cargo.[16]

Currently, there are roughly thirty injectable hydrogels approved for use in the clinic, with many more on their way.[10] Hydrogel delivery can allow for sustained release of a drug

product to gain a more extended advantage of a single administration, reducing the strain on patients. Vantas® is an example of a slow-release hydrogel currently used in clinics and is used for the prolonged administration of histrelin in advanced prostate cancer. It is a reservoir-based polymer system that allows for once-yearly administration of the hydrogel film, releasing 50 µg of histrelin acetate daily. [19]

PEGylation is one of the most successful techniques for immune response evasion and is considered the gold standard for protecting biological drug products.[20,21] However, anti-PEG antibodies that cross-react with other PEGylated treatments have been reported.[20] Not only does this lead to a reduction in the efficacy of the current treatment but might also cause potential complications for future PEGylated medication use. For this reason, new alternatives for increasing stability are as relevant as ever. Hydrogels can provide a versatile option for protecting therapeutic agents. This protection of cargo can be the result of the many characteristics hydrogels can possess. Table 1 summarizes these characteristics that will be elaborated on further.

In this review, the focus will be on the properties that hydrogels can possess, that contribute to increased cargo stability(ies), in both chemical and structural stability.

Table 1: Overview of hydrogel characteristics that contribute to enhanced cargo stability

Potential Protective characteristic	**Drug/protein/cells encapsulated**	**Polymer nature**	**Reference**
Water mobility	trichlormethiazide	GelMA	22
	flomoxef	GelMA	22
Microenvironment	Procaine	CBM	23
	Arithromycin	CBM	24
	Vancomycin	PEG-p(HPMAm-lac$_{1-2}$)-HA	25
Preferential Ca^{2+} binding	Insuline	p(MAA-g-EG)	26
Confinement	Polynucleotides of poly(dA)/poly(dT)	AA/BAA	22
	lysozyme	AA/BAA	22
	horseradish peroxidase	AGAR	27
	β-galactosidase	AGAR	27
	recombinant uricase enzyme	PAEU-HSA	28
Crowding	β-galactosidase	AGAR, Dex	27
	horseradish peroxidase	AGAR, Dex	27
	Human serum albumin	Pluronic F127	32
Physical barrier	Insuline	P(MAA-g-EG)	26
	5-fluorouracil	PEG	29
	Pancreatic β-cells spheroids	GC-HA	30
Redox polymers protecting against O_2 damage	[NiFe]- hydrogenase from Desulvofibrio vulgaris MF	PEI-ATTCBP	34, 35
Masking active sites	Recombinant uricase enzyme	PAEU-HSA	28
Polymer interaction with viral envelope	hepatic cell line (HuH-7)	Alg	31
Protection against reactive oxygen species	mouse fibroblast L929 cells	mPEG-pMet	32

Abbreviation list table: **AGAR**: *Agarose*, **Alg**: Alginate, **ATTCBP**: *(1-(3-(acetylthio)propyl)-1'-(3-isothiocyanatopropyl)- [4,4'-bipyridine]-1,1'-diium iodide bromide,* **BAA**: *bisacrylamide*, **CBM**: *Carbomer*, **Dex**: *Dextran*, **GC-HA** : *Glyco chitosan-hylaronic acid*, **GelMA**: *Gelatin methacrylate,* **HSA**: *Human serum albumin*, **mPEG-pMet**: *poly(ethylene glycol)-b-poly(L-methionine)*, **PEAU** : *poly(β-aminoester urethane),* **PEG**: *Polyethylene glycol,* **PEG-p(HPMAm-lac$_{1-2}$)-HA** : *poly(hydroxypropyl methacrylamide lactate)-Hylaronic acid,* **PEI** : *polyethylenimine,* **Pluronic F127**: *(poly(ethylene oxide), PEO)$_{100}$–(poly(propylene oxide), PPO)$_{70}$–(PEO)$_{100}$,* **p(MAA-g-EG)** : *poly(methacrylic acid grafted with poly(ethylene glycol*

2. Chemical stability

Hydrogels have been shown to reduce chemical instability(ies) of encapsulated therapeutics through different effects. Chemical instability is defined as the generation of a new chemical entity by bond formation or cleavage through oxidation, hydrolysis or other degradation/clearance pathways.[33] This change in the chemical structure will often lead to loss of function of the cargo. Hydrogels can possess multiple characteristics that decrease this chemical instability, as summarized in Figure 1.

Physical barrier

Just like with most delivery systems, hydrogels provide a protective shell-like layer between the encapsulated moieties and the outside environment, leading to decreased potential physio-chemical or biological disruptions of the encapsulated cargo.[34] This physical barrier is one of the main characteristics that allow for the protection of encapsulated products during the delivery to the target organs. Hydrogels can be tuned to be responsive to different triggers. Temperature, pH, ionic and photo triggers are among the most common.[35] Network density can be specifically tailored to restrict interactions between parts of both the humoral and cellular immune systems to biological molecules encapsulated in the hydrogels, as schematically depicted in Figure 1A. This allows for a protective layer between the encapsulated cargo, degradation enzymes, and immune responses[16] while allowing low levels of diffusion out of the hydrogel. Inside the hydrogel, the cargo enjoys the effects of the stability increasing characteristics described later on in this mini-review.

After a trigger-induced decrease of network density, higher levels of diffusion will allow for the release of the cargo. This mechanism can be tailored relatively well to fit the desired release profile both in location and rate.[11] One example of this is complexation-based hydrogels that can be pH-sensitive. At low pH, these hydrogels have a high network density. Cargo like insulin being carried are protected against degradation enzymes and the harsh pH conditions of the stomach. Within the intestine, a higher pH environment, swelling of poly(methacrylic acid-poly(ethylene glycol)) hydrogels allows for insulin release, resulting in the possibility of oral insulin delivery. [26] This physical barrier is also capable of shielding other biological cargo, like viral vectors or cells, from immune responses such as neutralizing antibodies.[36,30] Kim et al. showed that encapsulation in glycidol chitosan-hylaronic acid hydrogel films could protect encapsulated pancreatic β-cells spheroids against physical stress, protease attacks and cell-cell interactions with natural killer cells.[30]Additionally, incorporation of adenovirus expressing β-galactosidase in fibrin hydrogels showed protection against loss of bioactivity without binding.[36]

Other examples of physical barrier protection include bilayered “gates” that can be designed. He et al.[37] showed that making one side of the hydrogel a bilayered combination of polyhydroxyethylmethacrylate (p(HEMA)) and poly(methacrylic anhydride-Ethylene glycol) (p(MAA-g-EG))allowed for the protection of the cargo in the stomach and release in the intestine. This is caused by the different swelling behaviours of the two polymers. In this case, pHEMA has a swelling ratio unrelated to pH and p(MAA-g-EG) shows a significantly increased swelling behaviour at higher pH. This causes the bilayered side of the hydrogel to fold out and allow for release of acid orange 8 and bovine serum albumine as model drugs. [37] Designing a hydrogel network that shields cargo until optimal spatiotemporal release is therefore one of the important characteristics that lead to increased availability of cargo therapeutics at the desired site.

Polymer interactions with the environment

Polymer interaction with degradation enzymes can explain the improved stability of drug molecules loaded into hydrogels(Figure 1B). Multiple enzymes such as trypsin have been shown to be activated by Ca^{2+} ions. The binding of Ca^{2+} to complexation-based polymers results in a depletion of Ca^{2+} in the environment.[26] This removal of Ca^{2+} from the enzyme environment, causes the enzymes stability to decrease, allowing cargo peptides to be protected against enzymatic degradation. This allows for the stabilization of the cargo while encapsulated and after release.[26] Yamagata et al. (2006)[26] hypothesized that electrostatic interactions between free MAA units with the gastric enzymes could play a role in the protection of encapsulated insulin. However, this interaction should be controlled and tailored since it can result in reduced nutrient digestion [26]

Polymers that make up the hydrogels can alternatively be tailored to contain antioxidant species like L-methionine based hydrogels.[32] Here, the polymer protects the encapsulated L929 cells from reactive oxygen species (ROS) like H_2O_2 by sacrificing itself as substrate. This makes hydrogels also viable as delivery systems for diseases with overproduced ROS.

Redox polymers can be used to protect sensitive enzymes against O_2 deactivation. The applied potential at the active site of enzymes can be controlled through a well-designed redox polymer due to control of the current through electron transfer between moieties in the redox polymer. As a result, the insulation of the enzymes restricts the oxidative stress on the enzyme. This allows for the use of sensitive enzymes that would typically not be viable due to fragility or oxygen sensitivity as with for example metalloenzymes used in molecular catalysis.[38,39] This protection effect has so far only been used in electrocatalysis. For future biological applications it is important to note that the spatial separation of enzymes was deemed a critical feature of the hydrogel-hydrogenase system, that might not translate directly to biomedical applications. [38]

Furthermore, alginate hydrogels have also been shown to protect embedded cells from virus infections.[31] Ionic interactions of the negatively charged polymer can inhibit different mechanisms in the virion life cycle. Ionic interactions of negative alginate chains in Ca^{2+} -alginate hydrogels with components of the viral envelope, inhibit infection by interfering with envelope-membrane receptor interactions or hindering viral particles in gel environment[31]

Microenvironment

The microenvironment within a gel can have a stabilizing effect on cargo (Figure 1C). A microenvironment of high viscosity is created through ion-pairing of the hydrogel backbone with the drug molecule. Consequently, enzymatic degradation is decreased through a higher pH and/or reduced reactivity of the drug molecules.[23] As a result of many drugs' maximal stability at pH < 7[40], most drugs will be more sensitive to OH^- degradation instead of H^+. Using an acidic polyelectrolyte as a polymer will cause a negative zeta potential in the hydrogel drug system. This negative zeta potential will cause attraction of H^+ and repulsion of OH^-. Jimenez-Kairuz et al.[23] showed that besides the ion-pairing of a protonated drug in the resultant low pH microenvironment, this will also cause a decrease in two main parameters of the OH^- catalysis, being the decrease in [OH^-] presence and reduced reactivity of positively charged protonated drug.[23] However, it can be speculated that the attraction of other positively charged ions such as K^+ , Na^{2+} and Mg^{2+} might increase hydrolysis dependent on these ions such as the K^+ dependent hydrolysis of p-nitrophenyl phosphate.[41]

The polyelectrolyte polymer also causes a microenvironment of higher viscosity, decreasing the kinetic energy of the attached

protonated drug.[23] Proteins and peptides prone to deamination have shown to exhibit increased stability in this higher viscosity environment of hydrogels.[25] Esteban et al. were able to show an increase of up to 27.1 times in the chemical stability of arithromycinm indicating the potential extent to which carbomer hydrogels can improve chemical stability.[24]

Masking active sites

Hydrogels have also been used in the incorporation of enzymes[42].Incorporation in poly(ethylene glycol)-poly(β-aminoester urethane) hydrogels can result in masking of the surface of the encapsulated recombinant uricase enzymes resulting in additional stability and lower degradation of encapsulated enzyme by environmental proteases.[28] Luchini et al.(2008) hypothesized that the electrostatic interaction of their affinity bait polymer with the proteolytic enzymes such as trypsin potentially results in the unavailability or steric hindrance of the active site of the proteolytic enzyme. Consequently, leading to an increased resistance of encapsulated lysozyme to proteolytic enzymes by preventing their binding to the lysozyme.[43]

Water mobility

Hydrolysis is one of the main degradation pathways of pharmaceutical products.[44] In hydrogels, water is present in different states.[22] Water can be tightly bound to the polymer chains causing unavailability through immobility.[45] On the other hand, water can also be unbound and freely available. The mobility of water plays a role in both chemical and physical stability of therapeutics (Figure 1D). [22,45] However, the mechanism through which free water influences drug hydrolysis depends on whether the cargo is directly hydrolysed through interaction with water or if it is base-catalysed. Yoshioka et al. showed that in hydrolysis through interaction with water, lower levels of free water result in a direct decrease in first-order hydrolysis of cargo. In base-catalysed hydrolysis, second-order hydrolysis in the presence of base is also related to water mobility due to higher levels of free water being correlated with lower microviscosity.[22] Hydrophilic polymers in the hydrogel system should, therefore, be able to provide a protective effect through tightly binding water molecules and increasing microviscosity. This reduction in free water would result in reduced hydrolysis, through one of the previously named mechanisms, providing a stabilizing effect. In contrast to this reduction in hydrolysis, lower water mobility has been shown to be involved in higher levels of aggregation of α-synuclein, presumably due to aggregation-prone conformations being stable for an extended period of time.[46] This shows that the protective effect of hydrogels is often a balance between multiple factors.

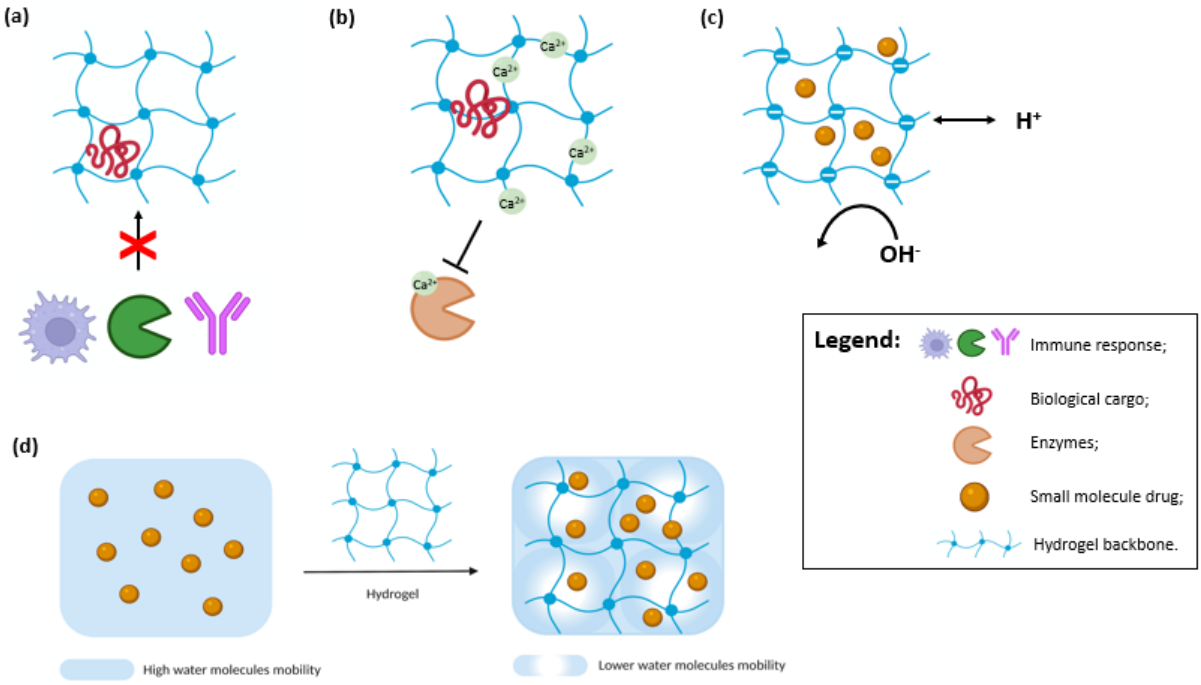

Figure 1: Graphical overview of hydrogel characteristics that can reduce chemical instability of cargo. ***A.****Physical barrier allows for protection of the cargo from immune response and degradation until desired location of release.* ***B.*** *Interactions of polymer with environment. Here the Ca^{2+} binding properties of complement polymers are depicted. Ionic interaction with Ca^{2+} results in depletion of Ca^{2+} in the environment causing degradation enzyme instability.* ***C.*** *Negatively charged microenvironment of a higher viscosity makes for a preferential environment for H^{+} degradation while most small molecules are degraded mainly through OH^{-} degradation* ***D****. Role of water mobility in hydrogels. In hydrogels, water is present in two forms: Bound water has a very low mobility, while free water can move and is responsible for hydrolysis. Hydrogels contain low percentage of free water through the tight binding of water by hydrophilic polymer chains and therefor reduce hydrolysis of embedded cargo.*

3. Structural stability

Hydrogels can have different effects on the structural stability of biological cargo some of which include proteins and peptides. The below mentioned characteristics have an influence on the cargo's activity and stability of its secondary, tertiary, and quaternary structure, not the stability of the hydrogel itself.

Crowding

Crowding within hydrogels can have a stabilizing effect on cargo, especially biologicals like enzymes and proteins. (Figure 2A). Environments with high concentrations of molecules that take up a large volume are found to stabilize the secondary structure of proteins. [47] This can either be done through the addition of other polymers in the hybrid gels that add molecular crowding (e.g., dextran chains)[27] or through the incorporation of other entities taking up a lot of space such as micelles.[48] The incorporation of dextran in agarose gels as a crowding agent to provide an hybrid hydrogels has shown to be very beneficial for horshradish peroxidase and β-galactosidase stability and half-life.[27]

Another example is Pluronic F127 hydrogels, they consist of immobilized micelles, allowing for only 50 % “void” in these types of hydrogels. Nandy et al. showed that bovine serum albumin incorporated in these Pluronic F127

hydrogels had increased secondary structure stability compared to free dissolved state in buffer.[48] The high degree of crowding ensures that the state of the protein which constitutes the least volume, is favoured. In proteins, the unfolded state takes up the most volume; therefore, the folded state is favoured providing an increase in secondary structure stability in proteins encapsulated in highly crowded hydrogels[49]

Confinement

Hydrogels can create a highly spatially-confined environment leading to increased conformational stability and increased resistance against thermal fluctuation.[18] Confinement and crowding, as mentioned above, are mechanistically different. Both these terms are often used to describe a restriction of solvent volume available for conformational dynamics of the protein. The lower conformational entropy state of the protein is often the correctly folded state due to it being more ordered, taking up less space and causing an higher entropy state of the environment. Unfolded proteins are highly unorganized and have exposed hydrophobic regions with interactions with surrounding molecules in the environment, leading to more options in the overall environment and therefor lower entropy of the environment.[50,51] There is a bias in nature towards higher entropy in a system.[52] Consequently, this state is favoured through both these mechanisms.

However, crowding and confinement differ in mechanisms through which they provide this effect. Crowding is volume exclusion by high concentrations of molecules that take up much space in solution. Confinement on the other hand, describes volume exclusion through a fixed and rigid structure such as a crosslinked 3D scaffold (Figure 2B).[50] Both these mechanisms can be used in hydrogel technology to increase structural stability but also decrease aggregation since this is often caused by the unfolding of proteins exposing the non-specific protein interaction sites.[50] Additionally, separation of the encapsulated proteins through these effects may play a role in reducing protein-protein interactions, therefore reducing aggregation.

However, for different proteins, crowding agents result in stabilization, destabilization, or no effect at all. This is likely through environmental chemical conditions that have to be balanced against the entropic and enthalpic stability of proteins caused by crowding and confinement. These chemical conditions are predicted to be a physiological tool cells used to tune protein stability[53]

Confinement has the potential to lead to increased thermal stability of proteins. The denaturation process is then hindered through the reduction of freedom in molecular motions.[54] This causes increased thermostability, pH stability and reusability of immobilized enzymes entrapped in these rigid hydrogels.[55] An example of this is esterase SulE encapsulated in poly (γ-glutamic acid)/gelatin hydrogel (CPE). Comparison between encapsulated esterase SulE and free esterase SulE after exposior to different temperature and pH conditions showed increased thermostability, pH stability and reusability. [55]

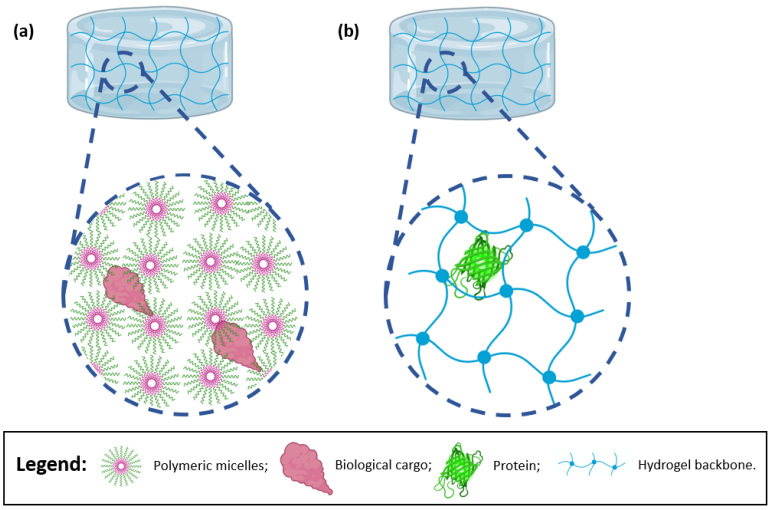

*Figure 2: Graphical overview of hydrogel characteristics that can reduce structural instability of cargo. Both of these describe mechanisms through which the lower entrophy state, in this case the folded state, are favoured **A**. Crowding is volume exclusion by high concentrations of molecules that take up a lot of space in solution. **B**. Confinement describes volume exclusion through a fixed and rigid structure*

4. Excipients for protecting cargo while encapsulating

Although hydrogels have shown the ability to protect cargo from degradation, it is also essential to protect the cargo during encapsulation. *In situ* encapsulation of molecules in hydrogels means that cargo is often exposed to reaction conditions such as Michael-type additions, radical polymerization, condensation reactions, Glaser couplings, cycloadditions, nucleophilic additions with reactive groups (like hydrazines,epoxides or azeradines) or Diels-Alder reactions as crosslink reactions.[56,57] This can result in a decreased degree of crosslinking in the hydrogel due to side reactions or loss in functionality of cargo (i.e. decreased release, loss of activity of pharmaceutical agent or increased immunogenicity) by covalently linking of cargo to polymer[58]. Having the crosslinking reaction happen under slightly acidic conditions, can reduce the nucleophilicity of the reactive groups on proteins. Hammer et al. showed that this is able to reduce crosslinking reactions with nucleophiles affected by pH such as Michael-type addition reactions. However, it does not affect cross-linking reactions unaffected by pH such as Diels-Alder reactions, and the acidic environment can potentially have a negative effect on protein stability.[56] It is desired to find different approaches to minimize these types of side reactions. One of these approaches is the addition of "excipients" to this encapsulation reaction. [58]

Polyanions

The addition of separate polyanions into the hydrogel encapsulation reaction has been shown to have a protective effect on proteins during crosslinking. This protective effect is driven by an electrostatic complexation of the seperate polyanion to the net positively charged protein surface, resulting in a cover around the protein preventing chemical reactions between protein and polymer chain, as shown in Figure 3. Multiple polyanions were screened (alginate, dextran, sulfate, heparin, hyaluronic acid and poly(acrylic acid))

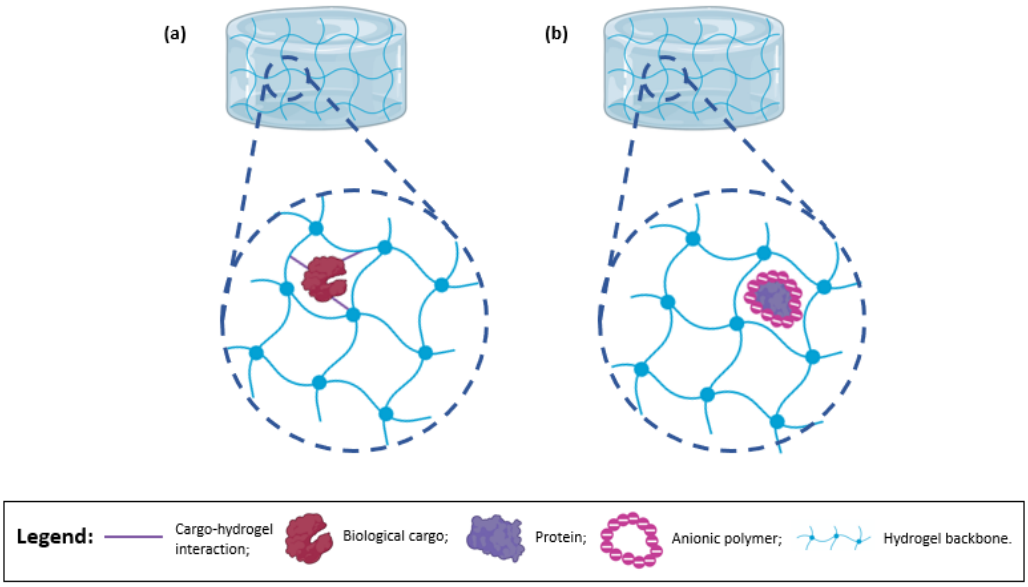

*Figure 3: In situ encapsulation often causes cargo to be exposed to a variety of crosslinking reactions. This can lead to covalent binding of cargo and polymer (**a**) leading to decreased release, loss of activity of pharmaceutical agent and/or increased immunogenicity. Complexation of polyanions and cargo prior to hydrogel crosslinking prevents covalent binding of cargo to hydrogel polymer by shielding the cargo causing it to be unavailable for reactions with the polymer chains (**b**).*

for their ability to protect lysozyme and bevacizumab as cargo in PEG hydrogels during crosslinking. One of the benefits of this technique is that it can be used under many different acidic, neutral, and alkaline pH conditions. After optimizing for specific polyanion and ratio, this allows for a great versatility in use.[58] This concept could potentially be extrapolated to also provide a solution for proteins with a net negatively charged surface if a polycation is used.

Precipitation of Cargo

Although precipitation can lead to aggregation, denaturation and difficulty to redissolve, it can also be used to prevent a reaction between cargo and polymer during crosslinking by precipitation prior to network formation. Linear PEG is often used to induce protein precipitation in protein purification from blood.[59] Its advantage lies in that it has shown to have little effect on denaturation or otherwise interact with the protein while decreasing protein solubility significantly.[59] Other, more specific methods of precipitation have also shown success. An example of this is Zn^{2+} addition to induce multimerization and precipitation in human growth hormone(hGH) prior to encapsulation. [60]

Radical scavenger & Chelator-divalent ion complex

Photopolymerized gels are created through radical polymerization in the presence of photoinitiators under light. Under visible and UV light, free radicals are formed allowing for the initiation of polymerization.[61] However, these highly reactive free radicals have shown to be prone to inducing side reactions between polymer and cargo during crosslinking. [62] The addition of vitamin C has been shown to increase the stability of DNA encapsulated in hydrogels. Vitamin C is a radical scavenger and neutralizes initiator radicals. A drawback of this is that only low concentrations of radical scavengers can be used since it would otherwise interfere with the hydrogel crosslinking.[63]

Furthermore, interactions of free radicals with proteins can result in loss of function or availability when loading proteins into photopolymerized hydrogels. This is caused by either conformational changes, aggregation, or covalent bonding to the support matrix. Certain proteins such as BSA have transition

metal-binding domains. It is hypothesized that these domains are also involved in the interaction between protein and polymer[62] Lin et al. (2006) [62]showed that the addition of a chelator complex i.e. iminiodiacetic acid (IDA), in the presence of divalent transition ions such as Cu^{2+}, can protect BSA during crosslinking.

This is likely through a mechanism where the IDA-Cu^{2+} complex prevents protein-polymer interactions in the reactive centre. However, it is also possible that the complex induces a conformational change that shields the active sites from free radicals. [62]

5. Conclusion

Progress in polymer chemistry and increased understanding of contributors to pharmaceuticals stability have allowed for the design of drug delivery systems capable of slowing a wide array of cargo instability. The versatility and ease of tunability in hydrogel design provide an excellent platform for drug delivery systems for specific applications. This tailorability also allows for the implementation of new and better ideas into hydrogels allowing for more specific and better protection of cargo. Future advances will only build upon the current body of knowledge regarding hydrogels, with hundreds of hydrogel formulations in clinical trials.

Abbreviation list: **AGAR**: *Agarose*; **Alg**: Alginate; **ATTCBP**: *(1-(3-(acetylthio)propyl)-1'-(3-isothiocyanatopropyl)-[4,4'-bipyridine]-1,1'-diium iodide bromide;* **BAA**: *bisacrylamide*; **CBM**: *Carbomer*; **Dex**: *Dextran;* **EG**: Ethylene glycol; **GC-HA** : *Glyco chitosan-hylaronic acid*; **GelMA**: *Gelatin methacrylate;* **HEMA:** Hydroxyethyl methacrylate; **hGH:** Human growth hormone; **HSA**: *Human serum albumin*; **IDA**: Iminiodiacetic acid; **MAA**: Methyacrylic acid; **mPEG-pMet**: *poly(ethylene glycol)-b-poly(L-methionine)*; **PEAU** : *poly(β-aminoester urethane);* **PEG**: *Polyethylene glycol;* **PEG-p(HPMAm-lac$_{1-2}$)-HA** : *poly(hydroxypropyl methacrylamide lactate)-Hylaronic acid;* **PEI** : *polyethylenimine;* **Pluronic F127**: *(poly(ethylene oxide), PEO$_{100}$–(poly(propylene oxide), PPO$_{70}$–(PEO)$_{100}$;* **p(MAA-g-EG)** : *poly(methacrylic acid grafted with poly(ethylene glycol;* **ROS**: Reactive oxygen species.

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