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Review

A Review on Recent Advances in Stabilizing Peptides/Proteins upon Fabrication in Hydrogels from Biodegradable Polymers

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Abstract: Hydrogels evolved as an outstanding carrier material for local and controlled drug delivery that tend to the shortcomings of old conventional dosage forms for small drugs (NSAIDS) and large peptides and proteins. Aqueous swellable and crosslinked polymeric network structure of hydrogels is composed of various natural, synthetic and semisynthetic biodegradable polymers. Hydrogels have remarkable properties of functionality, reversibility, sterilizability, and biocompatibility. All these dynamic properties of hydrogels have increased the interest in their use as a carrier for peptides and proteins to be released slowly in a sustained manner. The therapeutical peptide and proteins are remarkable therapeutic agents in today's world that allows the treatment of severe, chronic and life-threatening diseases, such as diabetes, rheumatoid arthritis, hepatitis in an easy manner. Despite few limitations, hydrogels provide fine tuning of proteins and peptides delivery with enormous impact in clinical medicine. The primary objective of this article is to review current issues concerned with the therapeutics peptides and proteins and impact of remarkable properties of hydrogels on these therapeutic agents. Different routes for pharmaceutical peptides and proteins and superiority over other drugs candidates are presented. The article will also review literature concerning classification of hydrogels on different basis, polymers used, release mechanisms their physical and chemical characteristics and diverse applications.

Keywords: hydrogels; peptides; proteins; crosslinked networks; controlled release; biodegradable polymers

1. Introduction

Targeted drug delivery to specific body parts has become one of the important ventures of the today world as conventional dosage forms are generally associated with difficulties in approaching the target site at the specified dose after or during a proper time period. As a result, the search for novel drug delivery systems and a new mechanism of action has become top of research areas. Novel drug delivery systems comprise of lipidic, proteic and polymeric technologies that provide a controlled and sustained drug delivery with better pharmacokinetics, stability towards the harsh external environment and avoid rapid clearance of drugs. Many of these advances have reached the market therefore as new drug carriers provide benefits [1]. Many drawbacks are associated with conventional drug delivery system which involves: Poor patient compliance; that occurs as a result of missing the frequent doses of drugs with a shorter half-life. A typical sawtooth pattern of plasma concentration-time profile is observed which makes attainment of steady-state concentration very difficult. There is also unavoidable fluctuations in the drug concentration that may cause under or overdose activity of drugs as the steady state concentration value fall or rise beyond the therapeutic range.

Recent research in advanced pharmaceutical preparations to provide stable and cost-effective drug delivery systems. The main concentration is on hydrogels that tend to reduce not only the shortcomings of old conventional dosage forms but also of novel drug delivery systems which needs a more convenient, compatible and stable drug delivery system for small drug molecules like NSAIDs (Non-steroidal anti-inflammatory drugs) or large molecules as proteins and peptides [2,3]. Hydrogels evolved as an outstanding carrier material for local and controlled drug delivery [4]. Hydrogels have been defined in many different ways by researchers over the past years. One of them, the most common are that hydrogel is an aqueous swellable and cross-linked polymeric network that produced as a result of the simple reaction of one or more monomers. The alternative definition also defines hydrogel as a polymeric material that possesses the capability of swelling and retains a certain high amount of water within its structure and do not dissolve in water medium itself. Considerable attention has been given to hydrogels over the past 50 years, due to their excellent performance in wide range of fields [5–7].

Hydrogels are also a part of nature on earth. Bacterial biofilms, usually aqueous extracellular matrix components and certain plant structures are ubiquitous water swollen components in nature. Agar and gelatin are also known ages due to its variety of applications early in human history. In 1936, DuPont's scientists published a paper on the synthetic methacrylic polymers. In his paper polymer as poly (2-hydroxyethyl methacrylate) (poly HEMA) was mentioned. It was mentioned as a hard, brittle and glassy polymer and it certainly it was not considered of much importance. After that work, poly HEMA was eliminated from consideration until 1960. Afterwards, Wichterle and Lim described the polymerization of HEMA and crosslinking agents in the presence of water and other solvents. This resulted in a soft, swollen, elastic and clear gel instead of brittle polymer. This novelty led to the advancement of biomedical hydrogels that are well known today. A large number of hydrogel preparations steadily grew over the years after this innovation and hydrogels were first successfully used contact lenses [8]. The intrinsic ability of hydrogels to absorb water is due to several functional groups (such as –NH2, COOH, –OH, –CONH2, –CONH–, and –SO3H) which are hydrophilic and attached to the polymeric chain. Hydrogels are resistant to dissolution and this property arises from cross-links between polymeric chains [9]. Hydrogels have remarkable properties of functionality, reversibility, sterilizability, and biocompatibility which meet fulfill material and biological requirements to treat targeted tissues and organs or replace it or interact with the biological systems [10–12].The important characteristics of these hydrogels are its ability to swell when interacting with water [13].

Hydrogels have various classification system as natural, synthetic or semisynthetic, according to the nature of crosslinking polymers. Hydrogels constitute chemical or physical crosslinking of polymers. Covalent bonds makeup chemical crosslinking while physical crosslink involves non-covalent interactions or crosslinked by a combination of both [14]. Synthetic hydrogels have replaced the natural ones owing to it advantageous properties. For biodegradation, sensitive bonds are introduced in the hydrogels backbone or crosslinks. These labile linkages are cleaved chemically or enzymatically in physiological conditions or by hydrolysis [15]. Biodegradation is the process of materials conversion into water-soluble components that are removed from the body without harsh effects. [16]. All these dynamic properties of hydrogels have increased the interest in their use as a carrier for peptides and proteins to be released slowly in a sustained manner from the hydrogel matrix and maintain a therapeutically effective amount in the surrounding tissues or in blood for a large duration.

The hydrogel matrix involves physical incorporation of proteins and its mechanism of release is controlled diffusion, swelling, erosion/degradation, or a combination of these. Hydrogels provide fine-tuning of the protein and peptide drugs release by redefining their cross-linking through changes in the polymer structure, its concentration, molecular weight, or chemistry. Of other methods to tailor drug release from hydrogel matrix are also known that involve reversible protein−polymer interaction or protein encapsulation in a secondary delivery system as micro/nanoparticles dispersed in the hydrogel matrix [17,18].

Hydrogels have enormous uses in clinical medicine and experimental settings for a wide range of applications, including tissue engineering and regenerative medicine [19], diagnostics [20], cellular immobilization [21], separation of biomolecules or cells [22], and barrier materials to regulate biological adhesions [23].

Although hydrogels have many advantageous properties, several limitations are also associated with these materials. They have a poor tensile strength which could limit their use in drug loading applications and can result in the premature dissolution or flow away of the hydrogel from targeted tissues and organs. This drawback is of much importance in many typical topical and subcutaneous drug delivery technologies. The amount and homogeneity of hydrophobic drugs are also minimized in hydrogels. Another disadvantage is the presence of high quantity of water and large pore sizes in most hydrogels which often result in relatively rapid drug release, in few hours. The application of hydrogel can also be problematic sometimes; though some hydrogels are ultra-deformable to be injected, many are not, thus need surgical insertion. Each of these issues critically limits the practical use of hydrogel drug delivery systems in the clinical applications [24]. Hydrogel innovation is also linked to problems like less solubility, high crystallinity, non-biodegradability; unfavorable mechanical and thermal properties, unreacted monomers and the use of toxic crosslinkers. Therefore, the improvement of these properties can be possible with the use of a combination of natural and synthetic polymers with pre-tuned properties like biodegradation, solubility, crystallinity and biological activities [25].

Peptides and proteins are known for years as complex structures. Naturally, there are twenty different amino acids join together peptide bonds and build chains known as peptides and proteins. The main difference between peptide and protein is that a peptide contains less than 20 amino acids, having a molecular weight less than 5000, while a protein has 50 or more amino acids and its molecular weight is larger than peptides. Several processes as fermentation, purification and recombination technology led to the production of potential proteinaceous drugs in an economical way which is used in wide range of diseases. They can be administered through various routes like oral, transdermal, nasal, pulmonary, ocular, buccal, and rectal. By making the availability of these pharmaceutical proteins and peptides possible these drugs can prove to safe and effective therapeutics. Due to its large applications in pharmaceutical fields, they will probably take the important place of organic-based pharmaceuticals. In the last years, therapeutic peptides and proteins have reached a successful level. Several diseases can be treated with this type of therapeutics include auto-immune diseases, cancer, mental disorder, hypertension, and certain cardiovascular and metabolic diseases. Recombinant technology has made possible the production of potential proteins and peptide drug it possible in a cost-effective way. This allows the treatment of severe, chronic and life-threatening diseases, such as diabetes, rheumatoid arthritis, hepatitis in an easy manner. Currently, over 160 protein drugs are available on the world market, and several hundred are on way in clinical trials. The total market of protein and peptide drug market has crossed 30 billion and expected to increase at 10% per year at least. The therapeutical peptide and proteins are have gained a place of important therapeutic agents rapidly. The peptides and protein-based drugs will be produced on a large scale by biotechnology processes and available on market for therapeutic use soon. The benefits of having favorable time to market and high level of success in clinical applications in comparison with conventional pharmaceuticals, therapeutical peptides and proteins will play the main role in the treatment of various ailments [26,27].

2. Pharmaceutical Peptides and Proteins

The term protein is derived from a Greek word proteios which means holding the first place [28].Proteins are polymers of 100 or more amino acids [29]. Protein is mainly composed of Carbon, Nitrogen, Oxygen and Sulphur molecules [30]. Proteins contain a linear chain of amino acids which are connected by covalent linkages called peptide bonds [31]. On the other hand, peptides are known to be the condensation products of alpha-amino acids. The alpha-amino group of one molecule and the alpha carboxyl group of another amino acid are condensed in peptides [32]. Regarding peptides two amino acids are condensed to form dipeptides, three join to forms tripeptides, four form

tetrapeptide and those that contain 2-20 amino acids are polypeptides [33–36].Every cell contains proteins and peptides that play a unique role in various biological functions. Recently the development of biological compounds and giant molecules like proteins and peptides have increased to a greater extent and have taken the place of organic compound used as pharmaceuticals [37]. Novel sustained release formulations of peptides and proteins provide better opportunities for the cure and prevention of disease. These formulations can affect the chemical integrity and three-dimensional structure of proteins during manufacture. Various polymers that are used for sustained release of these proteins and peptides the most important of which include poly (lactide-co-glycolide) (PLG) [38]. There is sufficient progress in the development of peptides and proteins as novel drugs and discovery, and this also imposes certain challenges on pharmaceutical developers to provide new and methods for therapies [39]. Generally, peptides are important and effective signaling molecules that bind to receptors on the cell surface, for example, G protein-coupled receptors (GPCRs) or ion channels, where they initiate intracellular functions. Their pharmacological properties represent them as best compounds for the manufacturing and design of novel therapeutics which are safe, tolerable and efficacious in humans. This feature represents the main factor of peptides as compared to traditional small molecules [40].

2.1. Peptides and Proteins Formulation and Stability Challenges

It is obvious that proteins and peptides have a major impact on health but there are still many current and future challenges that need to be addressed regarding the stability and formulation of proteins and peptides. These include:

2.1.1. Development challenges

The overall cost and time for the development of therapeutically active peptides and proteins have been increased immensely, that compel pharmaceutical scientists to focus only on those molecules that have greater chances of success in clinical trials. It takes approximately 10 years from the initial stage of discovery to their progress in into the pharmaceutical market, but the chances of success (POS) is not a guaranteed [41].

2.1.2. Safety and immunogenicity issues

Several pharmaceuticals modify the target site which has resulted in the competitive issue to choose the most effective therapeutic product among products of similar nature. Till date, there are five anti-TNF-α therapeutic proteins that are available in the market. These include Enbrel, Cimzia, Remicade, Humira, and Simponi. All these therapeutics are administered subcutaneously with the help of injection on monthly basis [42].Their safety and clinical efficacy are most important which can be achieved by careful selection of various factors including disease state, biological nature of the target, potency, safety margin, dosing, and selection of the patient population. The best therapeutical proteins can be accessed through its safety and clinical efficacy profile. The following are some factors that should be considered regarding safety and immunogenicity [43]:

1. Molecules must provide maximum therapeutic window
2. Route of administration
3. Longer half-life
4. Tissue distribution
5. Stability and enzymatic degradation
6. The solubility of molecule underdevelopment.

Immunogenicity is also the main challenge for drug efficacy and disease treatment [44]. Immunological reactions are inflammation, hypersensitivity, and mild skin reactions to severe anaphylaxis. These immune reactions are clinically controlled corticosteroids to subdue the inflammation, or by changing the dosing regimen. Recent advances in pharmaceutical biotechnology and genetic engineering improved the safety of proteins. Immune reactions resulting from the introduction of non-human antibodies are now replaced by fully human antibodies and the

humanization of rodent antibodies [45].Immunogenicity related to therapeutical proteins cannot be precisely predicted. Pharmaceutical scientists are focusing on discovery phase of these therapeutics to assess the immunogenicity and finally select a molecule with minimum immunogenicity as a successful clinical candidate.

2.1.3. Protein stability

Therapeutical proteins are most advanced and emerging class of pharmaceutically active drugs which are widely used in the clinical setup. However, the stability of these proteins is considered to be the major downside in making ideal clinical candidates [46].Therapeutic proteins usually aggregate when they are stored under high exaggerated conditions [47].Owing to this aggregation the therapeutical proteins display immune reactions and also decrease their overall activity [48].For this purpose, various strategies are applied to enhance the stability of therapeutic proteins [49].Among this first strategy is to change the amino acid sequences of the chain [50]. The second approach is the optimization of the formulation of therapeutical proteins [51].Thermosensitive polymers are considered to have a positive effect on the stability of therapeutic proteins. Biodegradable polymers are also known for the successful delivery of a variety of therapeutical agents. Stability of protein can also be increased by using nontoxic Nano-sized substances [52].

2.1.4. Protein degradation

Protein degradation has also been found as an important challenge during the discovery and development of therapeutical proteins. Aggregation is a major degradation pathway during the formulation and storage of these proteins. Outside their specific environment proteins tend to lose their stability. These conditions vary to a greater extent in cell compartments and extracellular fluids. If certain conditions are not observed during the storage, the therapeutical proteins may alter their activity. Therapeutic activity of proteins can diminish as a result of proteolysis, aggregation, and suboptimal buffer conditions. It is important to understand the degradation pathways in proteins and peptides such as oxidation, deamination, chemical cross-linking, disulfide modifications, and fragmentation [47].Several techniques are implemented to eliminate and prevent the degradation of therapeutic proteins. The most common of these include encapsulation within inert and biocompatible polymers. By using enzyme inhibitors degradation processes can also be prevented [53].

2.1.5. Metabolism and elimination

Hepatic metabolism and fast elimination is a major drawback in clinical uses of therapeutic proteins. Pharmaceutical scientists are trying to halt the first-pass metabolism. These struggles include the non-invasive administration of therapeutic proteins through such routes that bypass the liver metabolism. Moreover, injectable therapeutical proteins are also used to prevent the hepatic first-pass metabolism. The problem of short half-life can be overcome by encapsulating or making conjugation of desired therapeutical proteins with biocompatible polymers the increase their half-life. IL-1Ra is a naturally occurring anti-inflammatory antagonist of pro-inflammatory cytokines, which have been successfully encapsulated in PF127. This polymer provides sustained release and enhances the stability and therapeutical activity [53].

2.1.6. Protein–excipient interactions

Protein–excipient interactions are also known to cause stability problems and they must be evaluated during formulation and development to prevent any immunological reactions. Various strategies are used by pharmaceutical scientists to access the protein– excipient interactions. These techniques have been critically reviewed by Kamerzell et al. (2011) in the past couple of decades [53].

2.2. Advantages of Peptides over other Drug Candidates

Compared with other drugs and antibodies, peptides and proteins have the potential to penetrate further into tissues owing to their smaller size. Therapeutical peptides and proteins including synthetic have fewer immunogenicity problems than recombinant proteins and antibodies [54]. Other advantages over antibodies and other drug candidates include lower costs of manufacturing, superior activity per unit mass (15–60-fold, assuming 75 kDa for one combining site of an antibody and 10–50 amino acids for a therapeutic peptide), higher stability, less potential for immunogenic reactions and better organ or tumour penetration [55]. Therapeutic peptides have several other advantages over small organic molecules. They have greater efficacy, selectivity, and specificity in smallest functional part of the protein and only they bind to specific therapeutic target [56]. Also, the degradation products of peptides are amino acids are nontoxic, thus minimizing the risks of systemic toxicity [57]. Peptides have a short half-life and they accumulate in tissues and lessens the complications. Most natural therapeutic peptide activates the target receptors in very small quantity [58]. Some are receptor antagonists, which inhibit ligand-receptor interactions and these are marketed as well [55]. Following are some other advantages of protein and peptide in drug delivery system:

- Erythropoietin is a therapeutic peptide that is useful for production of RBC [59].
- The protein tissue plasminogen activator is another successful molecule used for heart problems such as stroke [60].
- Oxytocin found its application in the management of labor pain[61].
- Bradykinin tends to increases the peripheral blood [62].
- Somatostatin is applicable in stoppage of bleeding in gastric ulcer [63].
- Gonadotropin impel ovulation [64].
- Insulin maintains normal blood sugar level [65].

2.3. Proteins and Peptides Delivery Systems

Protein and Peptide drug delivery system is the novel drug delivery System. Proteins and peptides are found in abundant quantity in cells. They perform several functions due to their unique features as enzymes, hormones, structural element and immunoglobulin [66].The success of therapeutical peptide and protein involve they're targeted to the site of action. To achieve this goal scientists must consider several factors during the design and development of these therapeutics including clinical indication, pharmacokinetics, toxicity, and physicochemical stability of the drug [67].Drug delivery technology is a range of drug formulation and dosage form modifications that can be applied to existing drug substances with the aim to improve that drug's safety, efficacy, side-effect profile or patient compliance. These changes can benefit patients by making that drug more effective and tolerable. The delivery technologies for the application of pharmaceutical peptides and proteins include the following:

2.3.1. Pulmonary Delivery of Peptides and Proteins

Several routes of administration like parenteral (subcutaneous, intravenous and intramuscular) are used for proteins. However, the pulmonary route provides advantages over others as lungs possess a large surface area and an extensive vascular network that increase absorption. Inhalation delivery is also non-invasive which increase patient compliance over long-term use such as insulin for diabetes mellitus, or recombinant human deoxyribonuclease (rhDNase), also known as dornase alfa, for cystic fibrosis. Some of these inhalational peptides are shown in Table.1.

Table.1 Examples of proteins and peptides for inhalation [68].

Local diseases	Compound
a-1-Antitrypsin deficiency	a-1-Proteinase inhibitor
Asthma	Anti-IgE Mab Interleukin-1 receptor Interleukin-4 Lactoferrin Vasoactive intestinal peptide
Anti-tuberculosis vaccine	Muramyl dipeptide
Bronchospastic pulmonary diseases	Calcitonin gene-related peptide
Cancer/Pneumocystis carnii	Interleukin-2
Chronic bronchitis	Uridine triphosphate derivatives
Cystic fibrosis	rhDNase (approved product: Pulmozyme_) Secretin Targeted genetics adeno-associated virus for cystic fibrosis
Emphysema/Cystic fibrosis	a-1-Antitrypsin Secretory leukoprotease inhibitor
Idiopathic pulmonary fibrosis	Interferon-g
Lung transplant	Cyclosporine A
Oxidative stress	Catalase Superoxide dismutase
Respiratory distress syndrome	Surfactant-associated proteins from natural bovine lung extract (approved product: Survanta_)
Systemic diseases	Compound
Anemia	Erythropoietin
Anti-coagulation	Heparin
Cancer	Interleukins Luteinizing hormone-releasing hormone
Diabetes insipidus	1-Deaminocysteine-8-D-arginine vasopressin Desmopressin

2.3.2. Nasal Delivery of Peptides and Proteins with Chitosan and Related Mucoadhesive Polymers

Nasal delivery of peptides is an injection-free, non-invasive and painless way to self-administer drugs and provide rapid local and systemic drug action [69–71].In order to maximize patient compliance, the nasal route of administration is the focus of much attention as compared to the oral and parenteral route. Also, the nose to brain delivery can be a useful method to target central nervous system [69,70,72].

2.3.3. Transdermal Delivery of Peptides and Proteins

Transdermal delivery is the most competitive technology nowadays for the delivery of therapeutical proteins as an alternative to oral and injectable. One drawback includes the impractical passive delivery due to the large size and hydrophilic nature of these molecules. Therefore enhancement techniques are used to ease the delivery of these molecules through the skin. For example, the use chemical enhancers, iontophoresis, phonophoresis microporation and microneedles have shown considerable importance. Figure 1, 2.

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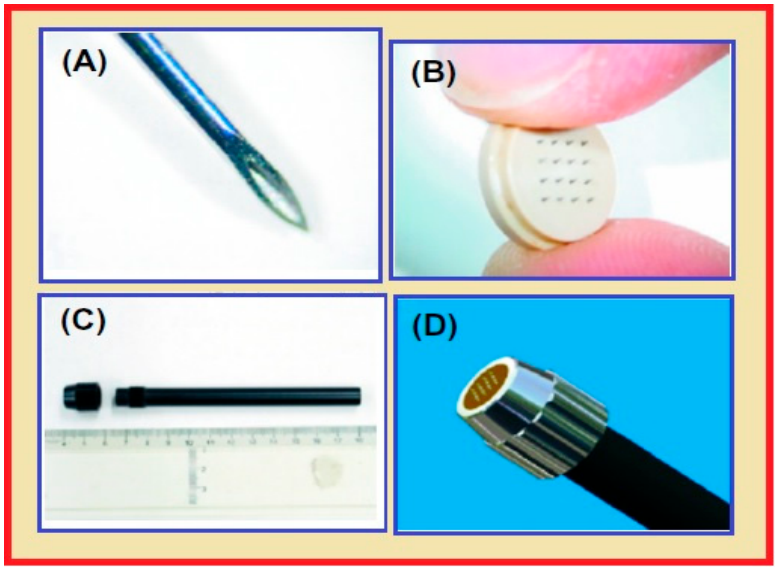

Figure 1. Microneedle array assembled from commercially available 30G hypodermic needles. (**A**) tip of a 30G hypodermic needle; (**B**) the assembled microneedle array; (**C**) microneedle array applicator in comparison with a ruler; (**D**) applicator at an angled view.

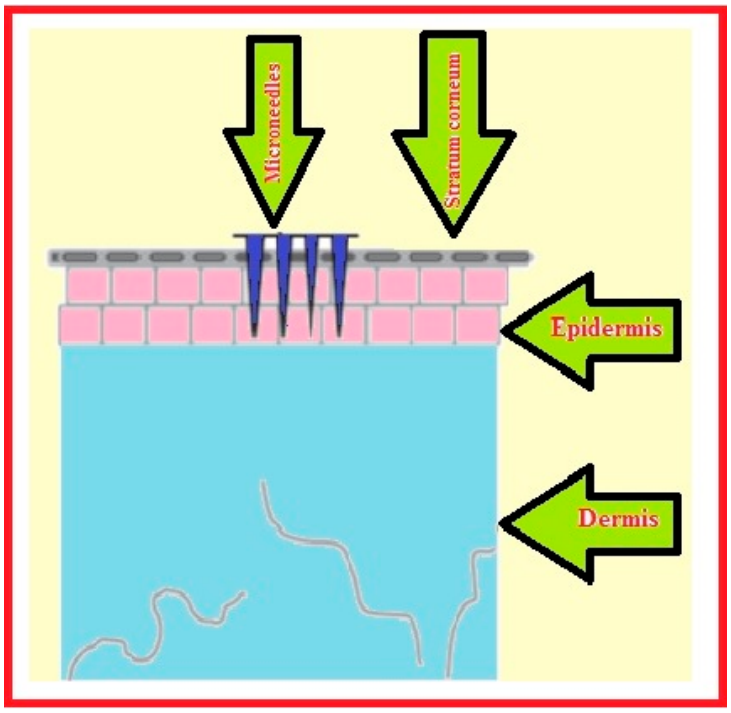

Figure 2. A schematic of microneedles applied on skin. Microneedles selectively penetrate the Stratum corneum and a part of the epidermis, thus allowing painless but efficient drug delivery.

2.3.4. Ocular Delivery of Peptides and Proteins

Protein and peptide delivery through the ocular route is an interesting technique. It involves several features to be considered for example physiological parameters of the eye and protective barriers which impose the main challenge for pharmaceutical technology [73].It is important to cross the protective barriers of the eye so that the therapeutic molecule can penetrate in optimal concentration to treat ophthalmic diseases or to exert its pharmacological action [74]. Conventional systems for ocular drug delivery include solutions, suspensions, gels, ointments and for drug delivery, but they have various problems such as poor drainage, tear turnover, poor corneal

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permeability, nasolacrimal drainage, systemic absorption and blurred vision [75]. Thus advanced drug delivery systems have been developed with targeted and controlled release properties to show successful results [73,76,77]. Till date, most of these therapeutic proteins and peptides that have been delivered to the eye have been for the treatment of local ocular disorders and some systemic disorders as shown in Table.2. Some of the shortcomings of ocular proteins and peptides are poor permeability, hydrophilic nature, enzymatic degradation and lower distribution. Nanocarriers and prodrug approach has been shown to overcome some of these limitations [78].

Table.2 List of disorders/indications where therapeutic peptides could be delivered through ocular route.

Disorder/Indication	Therapeutic peptide
Antiallergic, antiinflammatory	ACTH
Analgesic	b Endorphin, Leu-enkephalin
Antiscarring agent in glaucoma filtration surgery	Integrin-binding peptide
Attenuate miotic response	Somatostatin
Choroidal or retinal neovascularization	Octreotide, Urokinase derived peptide, Cyclic integrin-binding peptide
Corneal epithelial wound	Insulin-like growth factor derived peptide Substance P derived peptide
Diabetes mellitus	Insulin
Diabetes insipidus	Vasopressin
Diagnosis of thyroid cancer	TSH
Dry eye disease	Cyclosporine A
Hypoglycemic crisis	Glucagon
Immunostimulant	Met-enkephalin
Induction of uterine contractions	Oxytocin
Induction of vitreous detachment in vitretomy	Integrin-binding peptide
Paget's disease	Calcitonin
Secretion of insulin	VIP
Uveal melanoma and retinal blastoma	Apoptosis inducing peptide

2.3.5. Brain Delivery of Peptides and Proteins

During the last year's significant importance have been given to brain delivery. It requires the understanding of the mechanism of brain diseases. Various therapeutic proteins and peptides have been developed for the treatment of brain cancer and neurodegenerative diseases. However, certain barriers i.e. blood-brain barrier can be problematic in the delivery pathway. To overcome this problem, certain approaches have been developed to enable targeted drug delivery [79].

2.3.6. Chemically Modified Polyelectrolytes for Intestinal Peptide and Protein Delivery

Polyelectrolyte complex (PEC) formation is another advance system for peptides and proteins delivery. Conventional systems include nanoparticles made of polymers that are manufactured by solvent evaporation method which requires toxic chemicals of organic nature. This results in poor stability and biocompatibility [80–82]. These modified Nano-sized PEC are easy to manufacture and replace conventional Nanoparticulate formulations Figure 3. [83–87].

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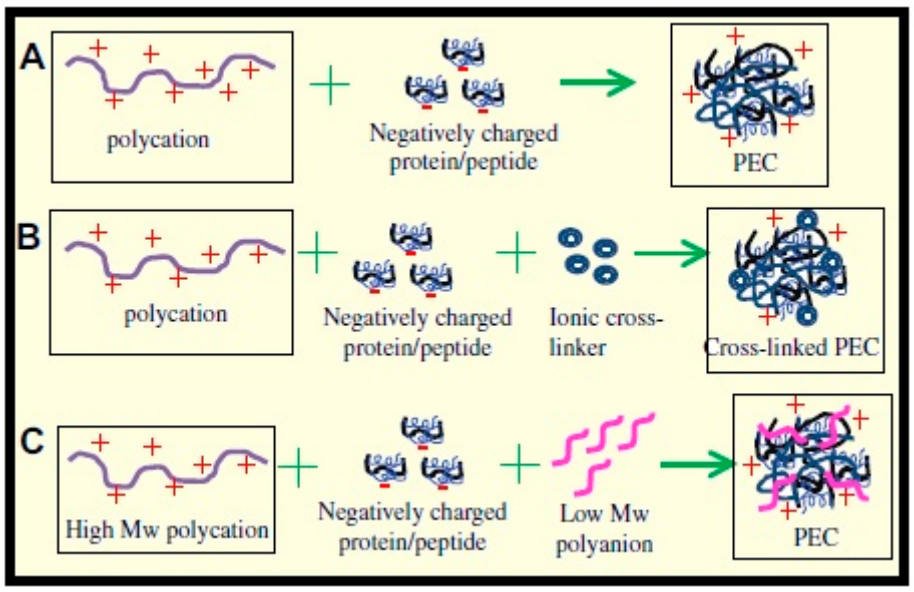

Figure 3. (**A**) Spontaneous formation of Nano-sized PEC occurs after mixing oppositely charged polymers and proteins in aqueous/buffer media. (**B**) Addition of an ionic cross-linking agent to stabilize the PEC. (**C**) Addition of another oppositely charged low molecular weight polyelectrolyte to form a stable PEC.

2.3.7. Nanoparticle-Mediated Oral Delivery of Peptides and Proteins

Oral delivery is a convenient method for peptides and proteins delivery. But this area represents a challenging goal. As therapeutical peptides and proteins can be formulated in the Nanoparticulate system, these can improve the bioavailability of protein drugs. Carrier systems for oral proteins are as important as the drug itself. Drug delivery in a controlled manner from nanoparticles can maintain the drug in therapeutically active concentration for prolong time thus influence the pharmacological activity. The advantages of these systems include increased patient compliance non-invasive method, minimum local and systemic side-effects, and thus a reduced toxicity profile. This also enhances the bioavailability of that drugs [88–91].

2.3.8. Peptide and Protein Delivery with Cell-penetrating Peptides

There are several obstacles in the effective delivery of peptides and proteins because of their hydrophilic nature. These obstacles are circumvented by using newer molecules that penetrate the cell in an efficacious way. They are also known as helper substances. Helper molecule like polyethyleneimine (PEI) is one of these agents. Other include encapsulating carrier systems such as liposomes or viral vectors, mechanical/physical membrane destabilizing techniques like electroporation or microinjection etc. [92–100]. They also have certain drawbacks, for example, heterogeneous dispersion, limited in vivo application, immunogenicity issues in the case of viral carriers [101,102]. Therefore a superior system is under research in 1988 a cell penetrating peptide called trans-activating transcription factor, Tat, from the human immunodeficiency virus (HIV) was discovered that had the ability to freely internalize the cells [103,104]. After this discovery, many efforts have been put develop these advance molecules as effective carriers. This vast research led to the growing field of cell-penetrating peptides (CPPs), also called protein transduction domains (PTD) or Trojan peptides because of their property to overcome plasma membrane and carry the attached drug into the cell.

3. Hydrogels

3.1. Hydrogels Classification

Hydrogels are classified on the basis of physical properties, swelling behavior, preparation method, origin, ionic charges, and sources, the rate of biodegradation and nature of crosslinking as shown in Figure 4 [105]. Among these two types of hydrogels are important, physical and chemical. In physical hydrogels, there is physical crosslinking that is attained through physical processes hydrophobic association, chain aggregation, crystallization, polymer chain complexion, and hydrogen bonding. While in chemical hydrogels chemical covalent crosslinking occurs to prepare the gel. Physical hydrogels are reversible due to the physical crosslinking whereas chemical hydrogels are irreversible because of changes. Another hydrogel is the combination of physical and chemical which is of dual nature with electrostatic interaction. It is used due to its ability to overcome the disadvantages of physical and chemical hydrogels. It is stable toward pH changes and other effects [106,107].

3.1.1. Types based on crosslinking

The introduction of crosslinking in hydrogel can impart some interesting properties in hydrogels. They show viscoelastic and sometimes pure elastic behavior. To have an idea of crosslinking in hydrogels, the different scientific approaches are explained based on the physical and chemical crosslinking as follows in the proceeding section: Figure 5.

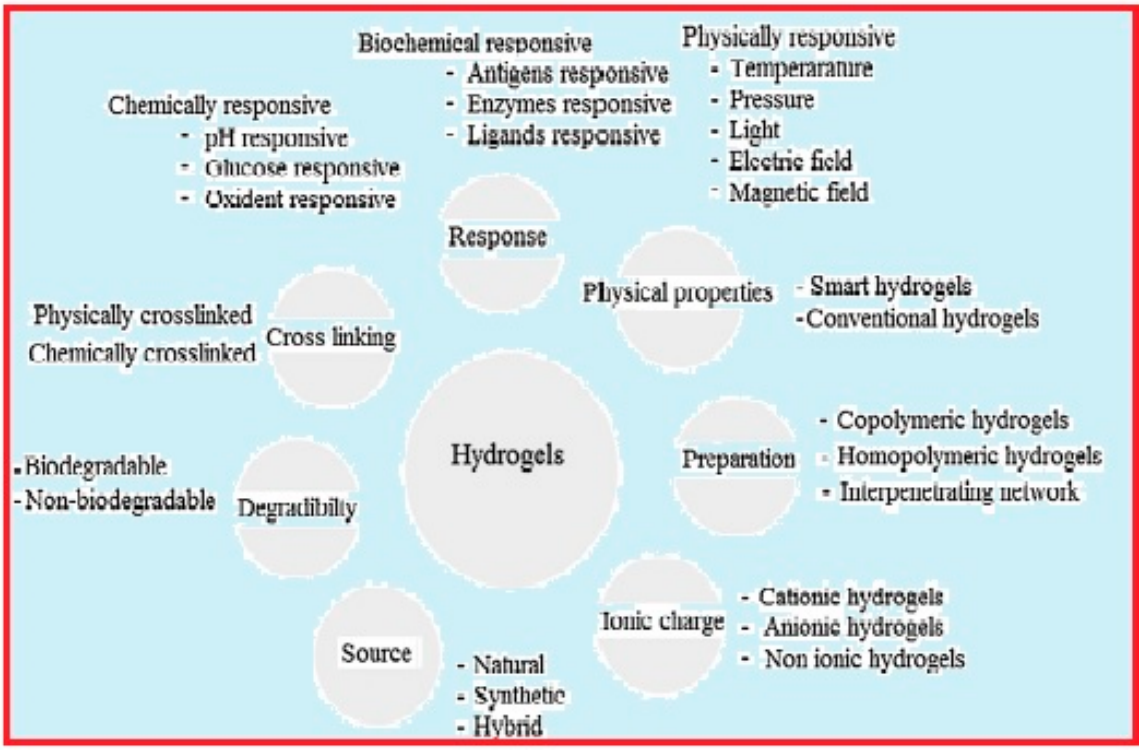

Figure 4. Classification of hydrogels based on the different properties.

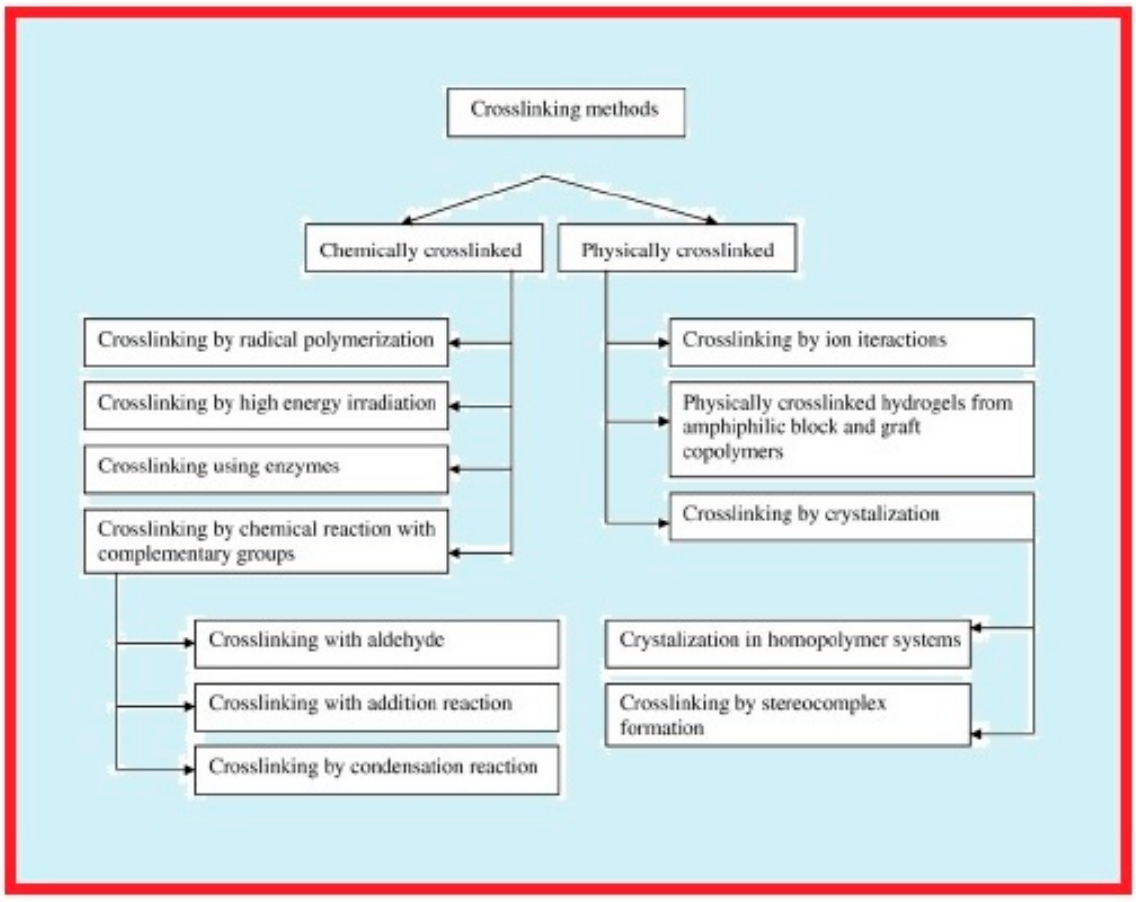

Figure 5. Novel crosslinking methods used in hydrogels.

A. Chemically cross-linked hydrogels

Chemically crosslinked hydrogels are irreversible with permanent covalent bonds exist between polymer chains. This property provides stability to the gel and cannot be dissolved in any solvents unless there is breakage in covalent crosslink points [107]. Chemical cross-linking results in a network with a relatively high flexibility and strength and due chemical bonding the degradation time extends. Chemical cross-linking can occur by various methods. Common of these include radical polymerization and chemical reaction.

I. Crosslinking by radical polymerization

Chemical crosslinked hydrogel have interesting properties of swelling and stimuli sensitivity that changes with the change in concentration of crosslinker. Radical polymerization can induce these properties in chemical hydrogels. Chemical hydrogels can be obtained by the radical polymerization of derivatives of water-soluble polymers with polymerizable groups. Different water-soluble polymers have been used for the design of these hydrogels via this route. They include both synthetic and semisynthetic polymers [108].

II. Crosslinking by chemical reaction of complementary groups

Chemical cross-linked hydrogels can be prepared by the use of water-soluble polymers with functional groups such as mainly OH, COOH, and NH2 which can be used for the formation of crosslinking in hydrogels. These functional groups react to form covalent linkages between polymer chains. Crosslinking by condensation reactions, crosslinking by addition reactions, and crosslinking by high energy irradiation and crosslinking by using enzymes have been identified for the chemical hydrogels [108].

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B. Physically crosslinked hydrogel

Physically crosslinked hydrogels have gained a lot of attention due to their unique properties such as their swelling and diffusion characteristics. Principally, the three-dimensional microstructure is very important because this microstructure plays a vital role in the stabilization of non-extracellular and extracellular matrices. Polymeric hydrogels have interesting characteristics such as soft tissue-like elastic, non-toxic, biodegradable and bio-comparable with stimuli sensitivity. Hence are widely used in several fields including drug delivery, self-healing, sensors, scaffolds, tissue engineering, diagnostics, immobilization, a coating of implants etc. Physical crosslinking can occur by several ways that are as follows: [109–111].

I. Crosslinking by ionic interactions

Physical hydrogels can be crosslinked by ionic interactions. For example, Alginate is a polymer that can be crosslinked by ionic interactions. It is a polysaccharide with mannuronic and glucuronic acid groups which are crosslinked by calcium ions [107]. Crosslinking by ionic interaction is done at room temperature and physiological pH which provide the ability to encapsulate living cells and proteins [112,113].

II. Crosslinking by crystallization

In such cross-linking polymer crystallizes to form a gel under specified conditions. For example, polyvinyl alcohol is a water-soluble polymer. When aqueous solutions of this polymer are kept at a certain temperature it turns into a gel but having low mechanical strength. If freeze-thawing is step applied during preparation, it forms strong and highly elastic gel [114]. The properties of such hydrogels depend upon polymer molecular weight, concentration in water, the temperature and time of freezing and the number of freezing cycles. Freezing is necessary for the formation of crystallites where physical crosslinking occurs. These gels are stable for 6 months at 37 °C [115]. Hydrogels formed by crystallization process have the water-absorbing capacity that provides the main area of an investigation by researchers interested in the basic properties of swollen polymeric networks. It also found applications in different technological areas. These include material for contact lenses and protein separation, a carrier for cell-encapsulation, as controlled release devices of drugs and proteins, a nutrient carrier for soil, cosmetics and increase oil recovery [108].

3.1.2. Classification based on method of preparation

On the basis of methods of preparation, hydrogels may be classified as homopolymers, copolymers, semi-interpenetrating networks and interpenetrating networks. Homo-polymer hydrogels are composed of one type of hydrophilic monomeric unit, while copolymer hydrogels have to crosslink of two co-monomer units, one of which must be hydrophilic to make it swellable. Interpenetrating polymeric hydrogels are produced by preparing a first network which is then swollen in the second monomer that forms an intermeshing network structure.

3.1.3. PH sensitive hydrogels

In such hydrogels stimuli of pH change is used to form a hydrogel. The pH range occurring at physiological, pathological, or subcellular sites such as the stomach, intestine, endosome/lysosome, and tumour sites is used for the formation of these hydrogels. Polymers used in this process are those with weak polyelectrolyte (polyacid, polybase) or polyampholyte sequences [116].PH sensitive hydrogels with ionic groups accept or donate protons with respect to changes in environmental pH change. The degree of ionization (pKa or pKb) changes in response to pH change changes. Two types of pH-responsive hydrogels are known: anionic and cationic hydrogels. Anionic hydrogels have attached groups of carboxylic or sulfonic acid. Here deprotonation followed by ionization occurs when the surrounding pH is above the pKa which induce swelling of the hydrogel [117–119]. On the other hand, cationic hydrogels have group's much as amine groups, where pH below the pKb is required for ionization and swelling [120,121].

3.1.4. Temperature sensitive hydrogels

Temperature sensitive hydrogels are unique to swell and shrink as temperature changes in the environment. Their swelling and deswelling behavior mostly is dependent on surrounding temperature. Temperature sensitive hydrogels can be classified as positive or negative temperature responsive systems [122].

I. Positive temperature hydrogels

Positive temperature hydrogels are hydrogels with specific upper critical solution temperature (UCST) [123]. Temperature above upper critical solution temperature is required for swelling of hydrogels. Whereas at a temperature below the UCST, dehydration occurs and the hydrogels shrink and release solvents or fluids from the matrix. Such types of hydrogels are retrogressive at negative temperatures. Positive temperature hydrogels shrink at low temperatures because of a complex structure formation by the hydrogen bonding.

II. Negative temperature-PHGs

This type of hydrogel is known by low critical solution temperature (LCST). Shrinkage occurs as temperature increases above the LCST and shows a swelling below LCST. The LCST is the most critical parameter for these hydrogels and can be altered in different ways including mixing of a small amount of ionic copolymer in the gels or by changing the composition of the solvent. Their hydrophobic constituent shifts them to lower temperatures [124]. The ratio of hydrophilic and hydrophobic constituents control the LCST. The hydrophilic part include –CONH–, and hydrophobic part is composed of –R–[125].Hydrogen bonds are formed at a temperature below LCST when water interacts with the hydrophilic part and this improves swelling and dissolution behavior. As the temperature rises above LCST, the hydrophobic interaction occurs and the hydrogen bonds become weaker that leads to shrinking of hydrogel [105]. Example of negative hydrogel includes PVP/PNIPAAM.

3.1.5. Glucose-sensitive hydrogels

These hydrogels respond to change in glucose concentration. For example, insulin hydrogel used for the treatment of diabetes responds to the glucose to initiate the release of insulin. It also contains glucose sensor suitable insulin delivery. Glucose-sensitive hydrogels are attractive insulin carriers with novel technology. Podual [126] and Brahim et al. [127].The swelling of hydrogel triggers the release of insulin when the local pH of the system reduces when glucose is converted to gluconic acid by glucose oxidase enzymes in the presence of oxygen. Glucose oxidase has been covalently attached to hydrogel network for controlling the release of insulin.

3.1.6. Protein-based hydrogels

Protein-based hydrogels are composed of special sequences, stereochemistry, and molecular weights. These are developed by using recombinant DNA technology for drug delivery and tissue engineering applications. One of best approaches for protein-based hydrogels includes coiled-coil method. In these hydrogels, the hydrophobic amino acid groups of the coiled-coil proteins are used in physical crosslinking of gels as shown in Figure 6. Physically crosslinked protein-based hydrogels are composed of tri-block copolymers with coiled-coil domains at the end and water-soluble polypeptide domains in the center [127]. By modifying the amino acid sequences in coiled-coil domain stimuli sensitivity to temperature and pH can be achieved Moreover 3D structure of the hydrogel is also possible when water-soluble linear synthetic polymer coiled-coil proteins are used as crosslinkers [128].

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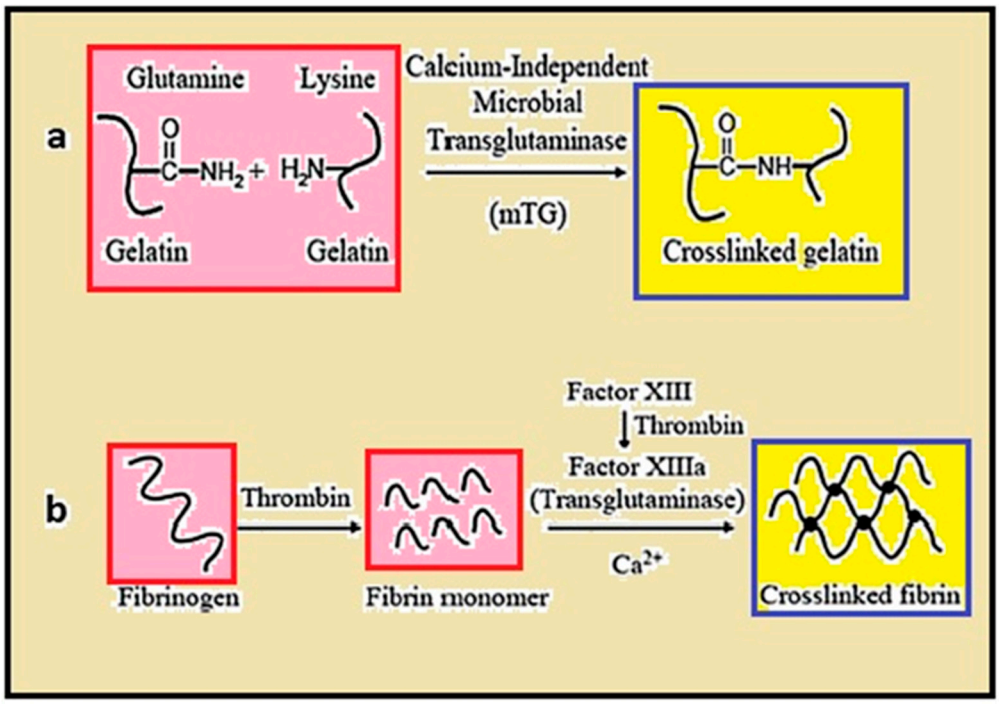

Figure 6. Protein-based hydrogels [129].

3.1.7. Antigen-responsive hydrogels

Antigen-responsive hydrogels are prepared by grafting antigens on hydrophilic polymeric backbones. These are used to deliver drugs at a specific target site [130]. When there is no free antigen so the chain antigen binds with the antibody and cause shrinkage of the hydrogel. They can be used as a useful carrier for biomolecules and deliver proteins at the target site through antigen sensing technique [131]. An antigen sensitive hydrogel is prepared by grafting antigen and the respective antibody to the polymer network, where they bind to initiate crosslinks into the network [130].

3.1.8. Smart hydrogels

Smart hydrogels are attractive new hydrogels that are composed of materials able to undergo transitional changes in response to environmental stimuli[16].These transitional changes include abruptly swelling, shrinkage, degradation, or they undergo a sol to gel phase transition when exposed to external physical or chemical stimuli, such as changes in pH, temperature, solvent, pressure, ionic strength, light, and concentration of specific biomolecules Figure.7[16].Environmental triggers provide specific functions, such as controlled drug release, protein separation, and muscle activity, or in situ gelling systems.

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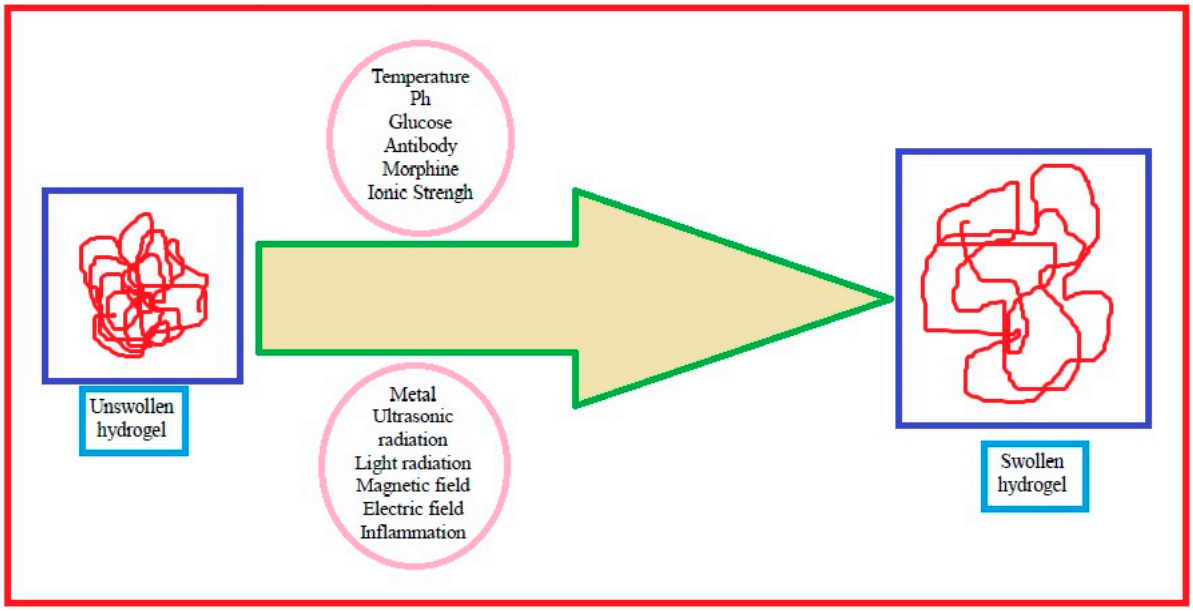

Figure 7. Representation of hydrogels stimuli-responsive swelling.

3.2. Monomers Used For Fabrication of Hydrogels

A range of monomers is used for the fabrication of hydrogels which include several novel materials with tailored characteristics suitable for particular applications. The first synthesis of hydrogel was carried out by Wichterle and Lin using PHEMA (Poly hydroxyethyl methacrylate) as the monomer. Monomers for hydrogel are selected based on their properties, ease of delivery or encapsulation as well as cost and availability considering their applications. One of the most common monomers used for drug delivery of protein from hydrogels is biodegradable PLGA (polymer of lactic and glycolic acid). However, PLGA is composed of hydrophobic materials and it denatures the proteins as well as cause inflammation due to degradation. By using hydrophilic polymers these problems can be overcome. For example acrylic acid, polyethylene glycol and methacrylic acid are all materials with hydrophilic nature, are used for therapeutic purposes. Researchers are now trying to synthesize materials suitable for specific applications. PNIPAAm (poly N-isopropyl acrylamide), PVA (polyvinyl alcohol) are all prepared by new techniques, for newer applications [8]. Table.3. Provide a list of common monomers used for biomaterial synthesis [25].

Table 3. Monomer used in the synthesis of hydrogel for Pharmaceutical Formulations.

Monomer Abbrevtions	Monomers
HEMA	Hydroxyethyl metharcylate
HEEMA	Hydroxyethoxyethyl metharcylate
HDEEMA	Hydroxydiethoxyethyl methacrylate
MEMA	Methoxyethyl methacrylate
MEEMA	Methoxyethoxyethyl methacrylate
MDEEMA	Methoxydiethoxyethyl methacrylate
EGDMA	Ethylene glycol dimethylcrylate
NVP	N-inyl-2-pyrolidone
NIPAAm	N-isopropyl AAm
Vac	Vinlyl accetate
AA	Acrylic acid
MAA	Methyl Acrylic acid
HPMA	N-(2-hydroxypropyl)metharcrylamide
EG	Ethylene glycol
PEGA	PEG acrylate

PEGMA	PEG methacrylate
PEGDA	PEG dicrylate
PEGDMA	PEG dimethylcrylate

3.3. Properties of Hydrogels

Hydrogels have received significant attention for their use in the pharmaceutical and biomedical applications. Hydrogels can serve as a carrier for drug and other therapeutic bio-molecules with biodegradable, biocompatible and non-toxic in situ properties. Once these materials are prepared they must be evaluated for the main properties like swelling behavior, mechanical strength and toxicity studies etc. so that the hydrogel can be safely and successfully used in the respective biomedical field.

3.3.1. Swelling properties

Hydrogels are composed of polymer chains linked together physically or chemically and thus, considered as one molecule irrespective of its size. Due to this reason, the molecular weight is not considered important in hydrogels and therefore, these are sometimes called infinitely large molecules or super macromolecules. They can respond to a small change in environmental conditions like pH, temperature, and an electric signal, the presence of an enzyme or other ionic species which cause reversible or irreversible changes. The alteration may alter the physical texture of the hydrogel. These changes can be macroscopic such as precipitate formation, size change and water content of hydrogels. The volume usually changes as the concentration of mobile ions in the hydrogel interior relative to the external solution (osmotic pressure) and pH fluctuate. Hydrogels with acidic or basic functional groups respond to the change in surrounding pH. Swelling nature and volume change depend upon the degree of ionization. For example, polyacrylic acid pH sensitive hydrogel where the ratio of swelling changes due to the ionization of carboxyl groups on the polymer chain [132].

3.3.2. Mechanical properties

Mechanical properties of hydrogels are very important for pharmaceutical and biomedical applications. The evaluation of the mechanical property is necessary for several biomedical fields e.g. soft tissue repair, dressing material in wounds, matrix carrier for drug delivery and cartilage replacement material. The mechanical properties of hydrogels are important for the maintenance of physical texture during controlled delivery of therapeutic agents to the target site. The desired mechanical properties can be achieved by varying the degree of crosslinking. A Higher degree of crosslinking results in strong hydrogel but it creates a brittle structure. Therefore the optimum degree of crosslinking is required to prepare a strong and elastic hydrogel. Copolymerization can also be used to achieve desired mechanical properties by forming hydrogen bonding [133].

3.3.4. Biocompatible properties

Hydrogels must be biocompatible and nontoxic to make it applicable for biomedical fields. Cytotoxicity and in-vivo toxicity tests are performed on hydrogels that must be within required limits. Biocompatibility is defined as the ability of a material to perform with an appropriate host response in a specific application. Biocompatibility has two basic two elements: (1) bio-safety that is appropriate host response not only systemic but also local, the absence of cytotoxicity, mutagenesis and carcinogenesis (2) bio-functionality i.e. the ability of a material to perform the intended function. Toxic chemicals of synthetic polymers is a challenge for in vivo biocompatibility. Toxicity can also be caused by initiators, organic solvents, stabilizers, emulsifiers, unreacted monomers and cross-linkers used in polymerization and synthesis of the hydrogel. For example, Irgacure 2959, is a type of photo-initiator that has been reported to decrease cell viability when used in concentrations over 0.1% [134].Purification methods should be used to avoid hazardous chemicals.

3.4. Loading of Drugs in Hydrogel

Drugs can be loaded in hydrogel matrices by two ways [135]:

a) Post-loading
b) In-situ loading.

First one is the post loading. In the post-loading method the polymer and drug are mixed first then initiators and crosslinker are added to polymerize the gel with the drug inside the matrix [136]. On the other hand in the in-situ loading, a preformed hydrogel is placed in a drug solution and allowed to swell till equilibrium. In both, the method the hydrogel is dried after drug loading. Drug loading within hydrogel is dependent on several factors including polymer and solvent interaction, cross-linking density of the polymeric network, the presence of a solvent etc. All these factors affect the ration of swelling to a greater extent [137]. The drug release from hydrogel matrix will be determined by mechanisms like diffusion, hydrogel swelling, reversible drug-polymer interactions or degradation of sensitive covalent bonds [135,138–143].

The final drug delivery device can be in the shape of:

- Thin film,
- Sphere,
- Cylinder,
- Irregular solid.

3.5. Release Mechanism of Drug from Hydrogel Matrices

Most of the drug release from hydrogel from hydrogels is through passive diffusion. Therapeutic moieties of different sizes and properties freely diffuse into/out of hydrogel matrix during the loading and storage of these gels. A hydrogel is hydrophilic which makes it highly different from non-hydrophilic polymer materials in consideration of the release behavior of the loaded drugs. According to various modelistic studies on the possible release mechanisms of an active agent from a hydrogel matrix, and considering the rate-limiting step of the release phenomena, the drug release mechanisms from hydrogels can be categorized as:

i) Diffusion-controlled
ii) Swelling-controlled and
iii) Chemically-controlled.

Diffusion-controlled is described by Fick's law (with constant or variable diffusion coefficient) as the most common and dominant mechanism for drug release [144]. Drug release by diffusion depends upon mesh sizes of the matrix material of hydrogel [145], which, in turn, is controlled by several factors such as the degree of crosslinking, chemical structure of the monomers and intensity of the external stimuli [144–146].Usually, mesh sizes from 5 to 100nm (in their swollen state) have been reported for biomedical hydrogels [146,147], which are much larger than most of the small-size drugs. These mesh sizes retard the release of several drugs while large macromolecules like oligonucleotides, peptides, and proteins show sustained release pattern. These mesh sizes can be custom design to allow the sustained release of macromolecules [148]. The second one is the swelling-controlled mechanism, in which diffusion of a drug is much faster. Swelling is considered to control the drug release behavior [149,150]. The last one is the chemically-controlled release where chemical reactions occur within the gel matrix. These reactions include breakage of polymer chain through hydrolytic or enzymatic degradation or reversible/irreversible reactions in the polymer network. These reactions tend to release the drug from the matrix. Several other mechanisms are also known for the controlled release of the drug. These include a surface or bulk erosion of hydrogels or the binding equilibrium among the drug binding moieties loaded within the hydrogels [25, 144,151]. The physical and chemical phenomena affecting the drug release kinetics include the following [143,152]:

- Wetting of the surface of drug delivery with release medium i.e. water.
- Medium penetration into the drug delivery system (e.g. via pores).
- Autocatalytic effects during hydrogel matrix degradation.
- Degradation of drug and polymer.
- Diffusion of drug or products of polymer degradation within the hydrogel matrix.
- Diffusion of drug or products of polymer degradation in the fluid.

- Dissolution of drug or degradation products of the polymer.
- Precipitation of drug or degradation products of the polymer.
- Surrounding pH changes inside the hydrogel matrix at the micro level, caused by the degradation of the polymer.
- Polymer swelling and closing of pores.
- Osmotic consequences caused by significant hydrostatic pressure.
- Acidic or basic microenvironments in the drug delivery device caused by degradation products.
- Physical drug-products formed by polymer degradation interactions (e.g. ion-ion attraction/repulsion and van der Waals forces)
- Chemical reactions between the drugs and degradation products of polymer or water.
- Convection processes as a result of significant hydrostatic pressure
- Adsorption and/or desorption phenomenon.
- Changes in the drug delivery device geometry or dimensions caused by shear forces[143].

3.6. Proteins/peptides Stability in Hydrogels

A successful drug delivery system depends upon the ability to release active proteins, as well as providing their sustained release. During the evaluation hydrogel-based system the assessment of protein stability must be implemented to verify pharmacological drug activity and the lack of immune reactions. The stability of the protein must be maintained during the preparation, storage, and release of the hydrogel. The major issue in hydrogel formulation includes maintenance of protein's structure due to incomplete release due to aggregation, chemical binding between protein and polymer, oxidation, deamidation, etc. [153]. Studies reported that oxidation affects the stability of bovine serum albumin (BSA) and interleukin loaded in hydrogels. The extent of oxidation can be reduced by the addition of an antioxidant. Post loading of proteins in gels can be used as a method to prevent interactions and to avoid their unwanted chemical modification [154]. Protein loading into polymer carriers can lead to loss of native 3D structure and denature proteins. Denatured proteins usually tend to aggregate. Their altered structure can induce immune reactions such as antibodies production that neutralize the activity of the therapeutic protein [155].Protein denaturation and aggregation have been found in protein/PLGA preparations [156]. As hydrogel have high water portion which provides better compatibility with proteins as compared to hydrophobic part like PLGA. Also, there is limited mobility of proteins in hydrogel matrices that further contributes to the stability of entrapped therapeutic proteins entrapped proteins. Many studies also reported that the structure and bioactivity are retained after it is released from the hydrogel. In general, hydrogels protect proteins from denaturation/aggregation more than other types of matrices. A number of supportive techniques are available to evaluate the structural changes of the protein, and many of them are used in combination to obtain a full characterization of the protein stability. Of these several techniques, High-performance liquid chromatography and size extrusion chromatography are used to determine possible changes in protein primary structure. These changes include oxidation, deamidation, or the presence of reversible aggregates. Mass spectrometry is also a useful method to investigate changes in the primary structure of proteins [157].Other methods for characterization of structural changes in pharmaceutical proteins, are Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), fluorescence spectroscopy, far and near-circular dichroism (CD) [158].

3.7. Hydrogels for Pharmaceutical Applications

Hydrogels are widely used as biomaterials in several applications due to their structural resemblance to the body tissues Figure.8. For example, drug delivery systems based on hydrogel is of great importance as these are tailored to provide a modified release to the target site, prolong circulation time, and reduce toxicity and side effects. Hydrogels not only serve as an excellent carrier for small molecules but also for delicate bioactive large molecules such as proteins. Due to its high water content, it retains the activity of proteins and protects from denaturation thus making it ideal

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encapsulation and release material for proteins. Hydrogels serve as ideal drug delivery systems with desirable therapeutic features [159].They have excellent physicochemical and biological properties with a wide diversity of polymeric materials which lead to excellent candidates for delivery systems of pharmaceutical agents [160–162]. Pharmaceutical hydrogels have various categories based on different criteria mainly including, route of administration [163,164], type of material therapeutic agent to be delivered [25,135,163], and release profile [165,166].

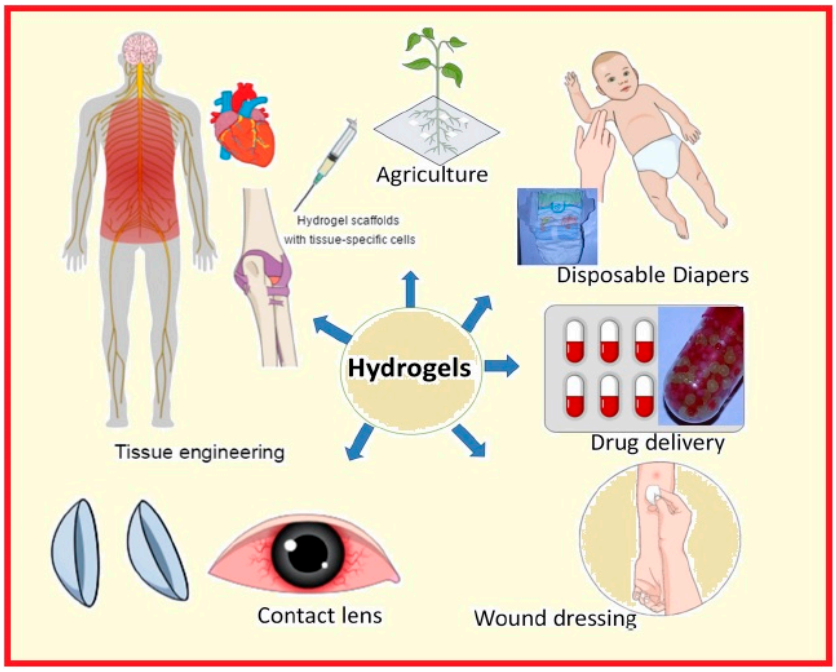

Figure 8. Hydrogels and their significance in their various fields of applications.

Following are some main applications of pharmaceutical hydrogels:

1. Superabsorbent hybrid hydrogels are found to have best water absorbent capacity which is useful in for plants growth. It can reduce consumption of irrigation water, retain fertilizer in the soil, lower the death rate of plants and increase plant growth. Studies reported that superabsorbent hydrogels are used as water-saving materials for the regeneration of dry and desert environments [167,168].
2. The presence of pollutants in the environment contaminates water at a high level and this polluted water causes immense environmental problems throughout the world. For this purpose superabsorbent hydrogels are used to remove toxic and hazardous heavy metal ions and dyes through adsorption mechanism. The adsorption mechanism is facilitated by functional groups in hydrogels including OH, NH2, CONH2, COOH and SO3 groups, which activate and modify the properties of hydrogels [169,170].
3. Special hydrogels are composed of superabsorbent materials and they are used as hygienic materials for various purposes such as in disposable diapers and lady napkins and absorb the secreted fluids [171].
4. Superabsorbent hydrogels are mainly used as targeted drug delivery system and Nano/controlled drug delivery systems.
5. Conducting polymer hydrogels (CPHs) are another unique types of hydrogels that are employed chemical industries, implants of electrochemical biosensors, and for electro-stimulated drug release [172]. CPHs have the ability as conductive flexible electrodes for supercapacitor purpose [173], also it is used in bioelectronics, server as energy storage devices and biosensors of glucose enzymes with high sensitivity [174].
6. CPHs are also used in battery industries such as lithium-ion battery technology and silicone anodes due to their magnificent electronic and electrochemical characteristics [175].

7. CPHs are unique types of polymeric hydrogels that found application in bioactive coating of electrodes and tissue engineering [176-178].It server as super biosensor material for various biological compounds such as enzymes, antibodies, nucleic acids, cells, etc. it has electronic transducer which is equipped with an electronic amplifier [175].
8. Polysaccharides based hydrogels for bioactive coatings (e.g. catheter, stent), replacement of nucleus pulpous and cellular scaffold (artificial organs)
9. Microporous hydrogels have the capacity for control release of therapeutic agents and for blood purification [179]. They are also used as regenerative medicine for cell delivery and dressing material or wounds [180,181].
10. Acidic cellulose–chitin hybrid gel that is a novel electrode which is used for electric double layer capacitor.
11. Hydrogels that are prepared from silk fibers prepared excellent interstitial fluid support capacity and they are employed for articular cartilage repair [182].
12. Elastin-like protein hydrogels are found to improve neurite out-growth on neuronal cultures. These hydrogels contain engineered polymers that produce elastin-like proteins recombinant protein containing amino acids.
13. Elastin-based polymeric hydrogels are mainly utilized for advanced engineering of elastic tissues, such as skin, lung, and vasculature. They are composed of expandable polymeric fibers which facilitate the blood vessels to stretch and relax billion times or more during their lifetime period [183].
14. Poly (hydroxyethyl methacrylate) based hydrogels are used to ensures good wound-healing therefore mostly applied in wound dressings, specifically burn dressings applications. It is used in contact lenses, drug delivery and tissue engineering purposes for bone marrow and spinal cord cell regeneration. It also promotes cell adhesion in artificial skin and cartilage manufacturing method [184].
15. PVA based hydrogels are used widely in biomedical applications including drug delivery, artificial tears, contact lenses, artificial cell encapsulation and as nerve cuffs[185].PVA hydrogel also found application in injectable implants, soft tissue fillers, cartilage reconstructive, aesthetic surgery, artificial organs, drug delivery systems and wound dressings. It provides a humid environment that is useful for wound healing [186].
16. Poly (ethylene glycol) based have high biocompatibility, lack of toxic effects on the surrounding tissues and high solubility in water which serve as good candidates for drug delivery systems[184]. They are employed for cell delivery to improve tissue regeneration.
17. Thermosensitive tri-block poly (ethylene glycol)-poly (ε-caprolactone)-poly (ethylene glycol) based hydrogels which are formed in-situ, can be easily utilized in various biomedical fields such as cell encapsulation, controlled drug delivery, and tissue repair. Poly (imide) (PI) hydrogels and PVA hydrogels are used in plastic and reconstructive surgeries [184].
18. Polyacrylate (PA) based polymeric hydrogels play important role in advanced aesthetic corrections, soft tissue fillers and enhancement materials [184].
19. Poly (urethane) hydrogels serve as drug matrices, artificial kidney membranes, and catheter coating materials [187].

4. Conclusion and Future Perspectives

Till date significant progress has been made in the field of hydrogels as functional biomaterial. Hydrogels form a promising material for controlled release of pharmaceutical proteins and peptides due to their capacity to incorporate therapeutical agents in hydrophilic polymeric network. Hydrogels provide fine tuning of proteins and peptides delivery with enormous impact in clinical medicine. Many crosslinking methods have been devised for hydrogel synthesis. These hydrogels are of huge interest for the encapsulation and entrapment of bioactive substances. Stimuli responsive and smart hydrogels form an attractive approach for non-invasive treatment. Their extraordinary characteristics enable them to be employed as essential tools in almost all fields such as biomedical, agricultural, industrial and environmental areas.

We further expect, an increase knowledge in the composition of hydrogel material will allow controlled release of more sensitive drugs. Hydrogel can be fabricated from nanosized particles termed as nanohydrogel, which is expected to provide improved stability to biopharmaceuticals such as peptides and proteins. Nanohydrogels can help to retain the three dimensional structure of these agents and direct them specifically to the target site. Nanosized hydrogel formulation can also be expected to minimize enzymatic degradation of peptides and proteins by protecting these agents in polymeric network. Hydrogel formulation with tuneable properties will provide patient specific treatment that would be highly promising for chronic diseases. There is a need to produce hydrogels with enhanced durability, improved mechanical properties and significant biocompatibility. Nanohydrogels with smart polymers are considered to fulfil all these properties in near future. More attention is needed to meet the specific requirements of advance drug delivery systems. Several challenges are still a part of advance hydrogel formulations, which have to be overcome for diverse clinical applications in coming years.

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