Indian Journal of Novel Drug Delivery

IJNDD

An Official Publication of Karnataka Education and Scientific Society

Review Article

Hydrogel System, a 'Smart' and 'Intelligent' Drug Delivery Device: A Systematic and Concise Review

PATIL JS*, GURAV P B, MANDAVE SV, JADHAV SM, KULKARNI RG

Department of Pharmaceutics, SVERI's College of Pharmacy, Gopalpur-Ranjani Road, P.B.No.54, Gopalpur, Pandharpur-413 304, Maharashtra, India.

ARTICLE DETAILS

Article history:
Received on 29 April 2014
Modified on 25 May 2014
Accepted on 03 June 2014

Keywords:
Hydrogels,
Biocompatible,
Stimuli materials,
Pharmaceutical applications

ABSTRACT

Hydrogels made from hydrophilic and three dimensional crosslinked polymers are discovered by Wichterle and Lim, since then they have been offered indispensible applications to the biomedical field including pharmaceutical sciences. As these systems have similarity with biological tissues more than any other class of synthetic materials and due to this hydrogels succeeded in attracting the attention of pharmaceutical scientists to explore their use in development of drug delivery systems for various bioactive molecules. The soft consistency, high water content and low interfacial tension with biological fluids of the hydrogel materials made them biocompatible. The unique property of the different sized molecules has an ability to diffuse in and out of hydrogels permitted their use as a carrier for drug delivery system. Further research on these systems resulted into invention of variety of hydrogel forms which are having versatile applications. There is an ampoule scope for preparing these systems as physiological stimuli materials so as to get the desired benefit as and when necessary. The present concise review emphasized mainly on the pharmaceutical and biomedical applications along with other properties of this unique system.

INTRODUCTION

Numerous advancements have been perceived over the last three decades in the field of pharmaceutical drug delivery research. Plenty of advanced drug delivery formulations have come in pharmaceutical applications with abundant of benefits such as cost effective production, maximum therapeutic efficacy, stability and significant improvement in the patient compliances. Past 40 years has witnessed a greater attention which has been paid on focused development of controlled and sustained drug delivery systems. The ongoing research programs in advanced drug delivery systems, not only focus on such advanced formulations which can overcome the problems associated with conventional dosage forms, but also on their biocompatibility, stability and convenient use. Controlled drug delivery systems are one of the resultant products of such research activities possessing great applications to overcome the shortcomings of conventional formulations.

Author for Correspondence:
Email: pharmajspatil@gmail.com

Although significant progress has been made in this field, more advances are yet to be achieved for the treatment of many clinical disorders. In these cases, it is also imperative to modulate the drug delivery pattern based on the necessity to provide the drug constantly in the system. Such drug delivery systems usually do not simply release the drug at some predetermined rate, but they do so in a pattern that the pharmaceutical scientist desires. For instance, insulin is delivered only when the blood sugar levels elevated. [1], calcitonin directly delivered to upper small intestine bypassing the stomach and macromolecules and genetically engineered molecules can be delivered across tissues at required rate. [2] Hydrogels as advanced drug delivery systems are designed to release the drug at zero order kinetics that ensures constant drug release for extended period of time. Drug targeting to the diseased cells can also be achieved by using some antibodies without affecting the normal cells. [3, 4]

Polymeric systems that modify their structure and in consequence their properties in response to changes in the physical and chemical characteristics of the physiological medium are

very promising candidates for gaining optimum control over the moment and rate of drug release. The above approaches of drug delivery designs can be achieved by the use of biocompatible, biosensitive carriers such as hydrogels. Hydrogels possess the unique water swollen and crosslinked feature, acts as biomedical carriers for the delivery of drugs, proteins, peptides and enzyme conjugates. Because of their network structure and thermodynamic property hydrogels decide the drug diffusion pattern by changing their molecular mesh size in response to external stimuli which makes molecular stability of the incorporated bioactive agents. The present review addresses the unique features, characteristics and versatile applications of hydrogel drug delivery system. Hydrogels are three-dimensional, polymeric networks with hydrophilic nature and capable of imbibing large amount of water or biological fluids. [5, 6] The networks consist of monomers or co-polymers being crosslinked and are insoluble in water. [7, 8] These network structures possess water holding capacity which is attributed to the presence of hydrophilic groups viz. amino, carboxyl and hydroxyl groups in polymer chains. The capacity of water retention in hydrogels depends upon the number of the hydrophilic groups and crosslinking density. The extent of water holding varies from 10 to 1000 times of the weight of the hydrogels. [9-11] The network structure of hydrogels may be macroporous, microporous or nonporous and such hydrogels release the drug from their pores through mechanism that dependent on drug diffusion coefficient, porosity and tortuosity of the gel network. [12-14] Microporous hydrogels have small pore size and releases the drug by molecular diffusion and convection release. Nonporous hydrogels are the mesh like structures of macromolecular dimension releases the drug only by diffusion mechanism. [15, 16]

Overview of Hydrogels

Hydrogels are described as smart or intelligent gels when their sol-gel transition occurs at conditions that can be induced in living body. Hydrogels are formed by loosely held cross-linked three dimensional polymeric networks, which swell in water without dissolving in it. The most characteristic property of hydrogels is that they swell in the presence of water and shrink in the absence of water. In dried form, the swollen networks of the hydrogels collapse due to the high surface tension of the water thus swelling and shrinking, hydrogels preserve overall shape and integrity. Maintenance of three dimensional structures of hydrogels and physical or chemical crosslinking of polymer chains are essential. It is tricky to change the shape of the chemical gels due to the chemical bonds present in the polymer chains. On the other hand the shapes of chemical gels can be manipulated in which the polymer chains of the physical gels linked through non-covalent bonds such as Van der Waal interactions, ionic interactions, hydrogen bonding, or hydrophobic interactions. [17]

Types of Polymers Used In Hydrogels

Hydrogels based on natural polymers such as starch, chitosan and alginate attracted of researchers interest because of their unique advantages like abundance, non toxicity, biocompatibility, and biodegradability. Cellulose is one of the abundant natural polysaccharide, which is usually used as the hydrogel owing to its excellent biocompatibility, and biodegradability. Cellulose is insoluble in water however substituted cellulose derivatives such as methylcellulose and hydroxy propylmethyl cellulose possess water solubility. [18] Numerous synthetic methods have been reported including chemical crosslinking, graft copolymerization and atom transfer radical polymerization for the preparation of hydrogels containing cellulose derivatives. Among the aforementioned methods, graft copolymerization of vinyl monomers onto cellulose or its derivatives has been studied extensively and is usually carried out by various chemical initiators or by irradiation. An alternative approach for graft reaction has also been developed, which involves the introduction of carbon-carbon bonds onto cellulose. Vinyl monomers are readily grafted onto the vinyl derivatives of cellulose by solution polymerization. [19]

Chitosan is another natural polysaccharide extensively used as a polyelectrolyte complex forming agent in the preparation thermoreversible hydrogels without addition of crosslinking agent. Polyethylene grafted chitosan shows improved solubility in water and the gelation was found to be possible in physiological pH values and also used for controlled drug release *in vitro*. [20] Chitosan based hydrogels have been also investigated as potential cell carriers for tissue engineering applications. Dextran an important polysaccharide usually converted into enzymatically biodegradable by reaction with

maleic anhydride and resultant hydrogel made from such dextran is partially biodegradable and pH sensitive. [21] Xyloglucon is a cytocompatible polysaccharide exhibited thermally responsive behavior when more than 35% of its galactose residues are removed. [22] Xyloglucon gels have been used as a drug delivery carrier for various applications. [23] Gelatin a biodegradable and biocompatible biopolymer protein present in nature with thermoreversible properties. An aqueous gelatin solution solidifies at temperature below 25 ºC due to the formation of triple helices and rigid three dimensional networks and when temperature raised to 30 ºC the conformation changes from a helix to the more flexible coil rendering the gel to form liquid again. [24] Gelatin when combined with other polymers shows thermal gelation close to the body temperature thus useful in biomedical applications. [25]

Gellan gum is another polymer commercially obtained from broth cultures of aerobic bacteria. A concentrated water solution of gellan gum is warmed up initially to induce the gellan gelation. When the temperature decreased, the chains undergo a conformational transition from random coils to double helices. Double helices further rearrange to form ordered junction zones (sol-gel transition) and thus giving a thermoreversible hydrogel. Much stronger physical thermo-reversible hydrogels are obtained by the addition of mono and divalent cations to gel solutions. The physical gelation ability of this polysaccharide makes it as suitable structuring and gelling agent in foods and toothpastes and binder, as a sustained release matrix. Aqueous solutions of gellan are used as *in situ* gelling systems, mainly for ophthalmic preparations and oral drug delivery. Physical gellan hydrogels prepared using mono or divalent cations are used for the preparation of tablets, beads or microspheres. Interpenetrating polymer networks or co-cross linked polymer networks based on gellan and other polysaccharide systems have also been developed as drug delivery matrices. [26]

Properties of hydrogels

Crosslinking Ratio

Dynamic swelling ability of hydrogels mainly depends on crosslinking ratio of polymers and incorporation of more crosslinking agent enhances the crosslinking ratio. Highly crosslinked hydrogels swell less due to the presence of tighter structure when compared to lower crosslinked hydrogels which is attributed to hinder in the mobility of polymer chain. Swelling ratio of the hydrogels depends on chemical structure of the polymer. Swelling ratio of hydrogels with hydrophilic group is higher compared to those with hydrophobic groups thus minimizing their exposure to water molecule. Swelling of stimuli sensitive hydrogels depends on specific stimuli such as pH and glucose concentration.

Mechanical Properties

Mechanical properties of hydrogels play crucial role in drug delivery applications. A drug delivery system designed to protect sensitive therapeutic agents such as proteins, must maintain its integrity so as to protect them till the drug released from the system. The desired mechanical propriety of the hydrogels can be achieved by changing the degree of crosslinking and by utilizing copolymerization process by incorporating a co-monomer that contribute to hydrogen bond formation.

Cytotoxicity of Hydrogels

The leaching of untreated monomers, oligomers and initiators present in the hydrogels leads to toxicity during applications and hence it is vital task to understand the toxicological effects of various monomers used. In some cases, the relationship between chemical structure and toxicity of monomers has been studied extensively. [27] This problem of toxicity can be solved by various measures including modification of polymerization process and extensive washing of the resulting hydrogels. The use of initiators in the formation of hydrogels leads to the toxicity due to residual initiators and this can be avoided by preparing the hydrogels through gamma irradiation technique. [28-32] Toxicity of the hydrogels can be evaluated by cell culture methods and are known as cytotoxicity tests and the evaluation methods of toxicity studies include extract dilution, direct contact and agar diffusion.

Types of Hydrogels

Based on the nature of side groups on the polymers, the hydrogels can be grouped as neutral or ionic. Mechanical and structural characteristics of hydrogels classify hydrogels as affine or phantom networks. Additionally, hydrogels can also be categorized based on the methods of preparation as homopolymer or copolymer networks. Finally, they can be classified on the basis of physical structure of the networks as amorphous, semi-crystalline,

hydrogen bonded supermolecular structures and hydrocolloidal aggregates. [33-37] Hydrogels may also be known as stimuli based as they show swelling behavior which depends upon the external environments and the drug diffusion is mainly based in turn on swelling behavior. The polymers employed in the preparation of stimuli responsive hydrogels are physiologically responsive, where polymer complexes can be broken or the networks can be swollen as a result of the change in external environments. Some of the factors which affect the swelling of stimuli responsive hydrogels include pH, ionic strength, temperature and electromagnetic radiation. [38]

Stimuli sensitive Hydrogels

Numerous polymers possess gel-sol-gel phase change property which may be responsible for diffusion of the drug from the gel system that triggers the drug release in response to external stimuli. Hydrogels provide such sensor properties can undergo reversible volume phase transitions or sol-gel phase transition upon change in the environmental conditions. This kind of intelligent or smart property of polymers play an important role in the drug delivery since they may dictate not only where a drug is delivered but also when and which interval it is to be released. [39] Stimuli that induce responses of the hydrogels include physical stimuli such as temperature; electric fields, light, pressure, sound, magnetic fields; chemical stimuli like pH, ions and biological/biochemical stimuli include bio-molecules. The functions of stimuli responsive gels can be roughly classified into three categories viz. mechanical motion, mass transport and conversion and transmission of information. [40] They can be used in controlled drug delivery system.

Temperature sensitive hydrogels

Temperature sensitive hydrogels are probably extensively explored stimuli sensitive polymers in drug delivery research as these hydrogels are able to swell or de-swell by the influence of change in the temperature of the surrounding fluid. They are two of types, negatively and positively thermosensitive reversible gels. Negative thermosensitive hydrogels have a lower critical solution temperature below which the polymers swell in the solution due to the hydrogen bonding between polymers and water molecules and above it they contract due to the domination of entropy. When hydrogen bonds are broken by thermal agitation, aggregation takes place eventually resulting in shrinkage of the thermosensitive hydrogels with increasing temperature. [41] A positive temperature sensitive hydrogels have an upper critical solution temperature and below it they contract. Certain interpenetrating polymer network hydrogels swell at high temperature and shrink at low temperature. [42] In general, these hydrogels made from hydrophobic polymers which show variable networks in response to temperature thus modulate the drug release. The aforementioned thermosensitive gels are specific, controllable, biodegradable and biocompatible modulated drug delivery devices. The drugs from anticancer, antidiabetic, hormones or proteins and peptides category are widely explored for such devices. Sometimes the gels are formed *in situ* and are particularly used for tissue targeting to the inflamed or diseased areas. [43, 44] Drugs like insulin, heparin, and indomethacin have been delivered using such types of hydrogels. [45]

Electric signal sensitive hydrogels

Electric current can also be used as an environmental signal to induce responses of hydrogels such type of hydrogels are usually made of poly electrolytes. Electro sensitive hydrogels undergo shrinking or swelling in the presence of an applied electric field. For instance matrices of chitosan were utilized as electrically modulated drug delivery system. [46] In electrification studies, release time profiles for neutral, anionic and cationic drug molecules from hydrated chitosan gels were monitored in response to different milliamperages of current as function of time. This concept was best utilized as potential matrices for the electrically controlled delivery of proteins and peptides. [47] When microspherical hydrogel particles placed in water without any salt, an applied electrical field causes shrinkage of the hydrogels due to electro osmosis and electrophoresis from the hydrogel to the cathode. [48]

Photosensitive hydrogels

Light sensitive hydrogels have potential applications in the biomedical field specially in optical switches, display units and ophthalmic drug delivery devices. These hydrogels may possess special advantages in the modulated drug delivery field over others because light stimulus is imposed instantly and drug will be delivered in specific amounts with high accuracy as the sensitivity of hydrogels is rate limited by thermal diffusion. Due to the advantages

mentioned earlier and instantaneous delivery capacity of these hydrogels attracted the attention of both engineering and biochemical fields. Photosensitive artificial muscles can be developed using light sensitive hydrogels or cartilage tissue engineering uses *in situ* forming gels, where the gel may undergo transdermal photo polymerization after subcutaneous injection were found to be applicable for drug release devices. [49] A novel tissue adhesive technology based on photo crosslinkable gelatin was developed that allows *in situ* drug incorporated gelatinous gel formation on diseased tissue and releases the drug in sustained manner. [50] The same concept was also utilized for barriers and local drug delivery in the control of wound healing. [51]

pH sensitive hydrogels

pH sensitive hydrogels have been extensively used in the development of oral controlled release formulations. The acidic pH of stomach elicits pH dependent behavior of polyelectrolyte hydrogels as they swell minimum in a neutral pH. Polymers used in pH sensitive hydrogels contain pendent acidic or basic groups that either accept or release protons in response to change in surrounding pH. This property has been used to prevent release of drugs which possess foul and bitter taste in the neutral pH. Hydrogels made of poly anions crosslinked with azoaromatic crosslinkers were developed for colon specific drug delivery due to the low swelling capacity in stomach and more swelling in increased pH of intestinal tract which is attributed to ionization of carboxylic groups. These hydrogels can only be degraded in the colon by azoreductase enzyme produced by the microbial flora of the colon. [52, 53] The polyelectrolytes with large number of ionizable groups are used as polymers in such hydrogel preparation. As the external pH increases the swelling of hydrogel also increases in the case of weakly acidic groups, but decreases in polymer contains weakly basic groups. These hydrogels swell in water depending upon pH prevalent in the external environment. pH sensitive hydrogels have been used to encapsulate proteins in acrylamide polymer crosslinked with bisacrylamide acetal. At pH of around 5, the pore size of the acetal crosslinked hydrogels increases leading to release of protein, but at neutral pH these groups remain intact and protein do not diffuse out easily. [54, 55]

Ion sensitive hydrogels

Some of the polysaccharides employed as polymers to produce ion sensitive hydrogels may undergo phase transition in presence of various ions. [56, 57] Carrageenan form gels with some cations whereas Gellan undergoes *in situ* gelling in presence of mono and divalent cations. Low methoxy pectins form gel in presence divalent calcium cations and alginic acid undergoes gelation in presence of divalent and polyvalent cations due to the interactions with guluronic acid blocks in alginate chains. The phase transition behavior of positively charged hydrogels is sensitive to concentration of sodium iodide at the critical concentration which induces hydrogels to collapse state phase. [58] This unique phase transition behavior could be applicable for the formation of ion sensitive biosensors.

Pressure sensitive hydrogels

Some of the hydrogels may undergo pressure induced volume phase transition based on uncharged hydrogel theory of thermodynamic calculations. As per this theory hydrogels collapse at low pressure and expand at high pressure. The poly (N-isopropyl acrylamide) hydrogels swells under static pressure when the temperature closes to its critical solution temperature. [59] The pressure sensitivity appeared to be a common characteristic of temperature sensitive gels and it can be concluded that the pressure sensitivity is due to an increase in their low critical solution temperature value with pressure. [60]

Glucose-sensitive hydrogels

Modulation of insulin drug delivery is the real challenge in the area of development of self regulated insulin delivery systems. Design of delivery device of any other drugs is quite simple as compared to the delivery device of insulin, as insulin has to be delivered in a precise amount at the exact time of need. Thus, it is required to develop modulated insulin delivery system which possesses the glucose sensing ability and an automatic close-off mechanism. The release and close-off of drug release mechanisms are regulated automatically based on the concentration of glucose present in the biological systems. Many researchers have attempted to develop modulated insulin delivery system in order to release the drug in response to the blood glucose concentration. Many hydrogels systems have been developed for modulating insulin delivery based on glucose sensor built in

system and are categorized into mainly three types.

Glucose oxidase loaded hydrogels

Glucose oxidase is the most widely used enzyme in glucose sensing. Combining this enzyme as a glucose sensor along with basic pH sensitive nature of the polymer, the system can be effectively used to regulate insulin release by sensing glucose. Primarily, this enzyme oxidizes glucose present in biological system and gives gluconic acid which leads to change in pH of environment. The aforementioned principle offers a wide scope for the selection of use different types of pH sensitive hydrogels which swell in the lower pH due to ionization and forms a hydrogel membrane facilitates release of insulin. [61, 62]

Lectine loaded hydrogels

Lectines are glucose binding proteins, which interact with glycoproteins and glycolipides on the cell surface that subsequently induce cell agglutination, cell adhesion and hormone like action. This unique property of lectine can be exploited for the fabrication of glucose sensitive drug delivery system. Therefore, researchers have reported glucose binding properties of concanavalin A (Con A, obtained from jack bean plant), a lectine possessing four binding sites. In this system insulin molecules are attached to a carrier through a specific interactions and this attachment can be interrupted by glucose itself. [63]

Phase reversible hydrogels

Sol-gel-sol phase transformation of hydrogels can be possible depending upon the nature of external environment with respect to glucose concentration and sol-gel reversible phase transformation demands glucose responsive crosslinking. Hydrogels which show sol-gel-sol phase transformation usually contains Con A which exists as tetramer at ambient pH. Each subunit represents the glucose binding site and specific interaction with glucose creates a platform to crosslink with polymer chains. The reversible nature thus resulted is attributed the noncovelent interactions between Con A and glucose. Once the external glucose penetrate hydrogels and diffuse into it, the free glucose molecules thus diffused compete with the glucose-polymer and resulting in exchange of glucose molecules with polymer attached glucose molecules. Hydrogels with phase transfer attribute show a great influence on insulin release, a varying diffusion of insulin in sol phase and gel has been reported and release of insulin can be managed by glucose concentration of the external environment. Con A is not compulsory for the preparation of phase reversible hydrogels. One of the phase reversible hydrogel has been generated using polymers which possessed phenylboronic acid groups in combination with polyol polymers. A complex formed between pendant pheylboronic acid group and hydroxyl groups of polyol polymer during gel formation. [64] Upon interaction with glucose, the hydroxyl groups of glucose compete with hydroxyl groups of polyol polymers for the borate crosslinking. Apparently, as the concentration of increases, crosslinking density decreases resulting in swelling of the gel thus facilitating the release more insulin and also gel converts into sol.

Protein Sensitive hydrogels

Some hydrogel based drug delivery devices developed as specific protein responsive systems which are highly desirable and useful in certain biomedical applications because of their high potential for tissue engineering and drug delivery systems. These systems can be categorized into two types.

Enzyme sensitive hydrogels

A concept of using specific enzymes which digest certain biodegradable Polymers, has been explored for the development of targeted or site specific drug delivery system. Enzyme sensitive hydrogels can be prepared from biodegradable polymers which can be digested by specific enzyme. Few specific enzymes were used as signals to diagnose and monitor several physiological changes in specific organs thus can be utilized for the development of site specific drug delivery. For instance, microbial enzymes specifically present in the colon can be used as signals for site specific delivery of the drug to the colon. [65]

Antigen sensitive hydrogels

Antigen and antibodies bind with each other at specific recognized sites through multiple non covalent bonds. The specific antigen recognition function of an antibody can provide the basis for constructing sensors with multiple uses for immunoassays. For release of antibiotics at the site and time of infection, poly (vinyl-alcohol) based hydrogels loaded with grafted gentamycin can be enzymatically degraded by thrombin. [66] This type of approach can be applied to occlusive

wound dressings and infection prone catheters. This system has sufficient specificity as an infection responsive controlled drug delivery system.

Molecular imprinting of hydrogels

Molecular imprinting is a technique to create biomimetic polymers possessing molecular cavities for molecular recognition. [67] Some proteins such as enzymes and antibodies can recognize specific substrate based upon the correct fit of guest molecules in their cavities through non covalent bonding. In molecular imprinting, some functionalized monomers are pre arranged around a print molecule through a non covalent bonding and then polymerized. [68] After this the print molecule is removed from the resultant polymer, resulting in a molecular cavity and thus recognizes the print molecule. In this approach most of the time a large amount of crosslinker is necessary to fix the structure of the molecular cavity in order to memorize the print molecule. [69]

Nanohydrogels

Some of the natural polymers yield hydrogels by self aggregation in water after sonication for appropriate time at desired conditions. The size of the resultant hydrogels can be controlled by varying the process conditions. These hydrogels can be prepared by using natural polysaccharides like dextran, pullulan, or cholesterol containing polysaccharides. The dimensions of such hydrogels are usually in the range of 20-30 nm and are used for cell targeting as they release the entrapped drug through pH stimulated response. Drugs like adriamycin has been delivered to tumor cells and delivery system showed pH dependent release and the highest release were reported below pH 6.8. [70] These nanoparticles of hydrogels have been used for controlled release of proteins like lysozyme, albumin and immunoglobulin.

Applications of hydrogels

Pharmaceutical Applications of hydrogels

Efficient therapy of any illness not only depends upon the dose, mode of administration but also depends upon the accuracy and promptness of a drug delivery system. A Number of strategies have been proposed to achieve drug delivery system for efficient therapy and among them, hydrogels have attracted considerable attention. Extensive researches have emphasized on applications of hydrogels in controlled drug delivery. Hydrogel based delivery systems are widely used for oral, ocular, rectal and parentral applications and also as excellent bioadhesive and targetable devices of therapeutic agents. In oral applications, drug loaded hydrogels can deliver drugs to mouth, stomach, small intestine and colon and bioadhessive nature can also be exploited for the release of drugs in controlled manner at desired site. Hydrogel based ointments may be employed for topical treatment of some diseases in the oral cavity and such hydrogels offer an excellent way to carry the liposomes. Specific utility of hydrogels have been mentioned for the drugs which need zero order release in a pulsatile fashion which is exemplified by delivery of insulin. Insulin delivery can be modulated by utilizing smart hydrogels which release insulin in response to the concentration of glucose in the surrounding environment. Microspherical hydrogels derived from sodium alginate demonstrated wonderful capacity to encapsulate the insulin producing cells. In numerous delivery applications, hydrogels remained a perfect choice to deliver drugs in topical applications due to their moisturizing property and such property has been extended to deliver synthetic corticosteroids for the treatment of inflammatory diseases instead of conventional creams. [71] Topical hydrogel based antifungal formulations of clotrimazole has been developed for vaginitis with a better absorption than conventional cream formulations. [72] Hydrogels made from polymers such as xyloglucan are utilized to incorporate pilocarpine and timolol as sustained drug delivery devices for ocular drug delivery. Drugs like diclofenac and phenaramine maleate have been effectively delivered through hydrogels for ocular conditions. [73, 74] *In situ* gelling system of alginate with high glucoronic acid has been used for ophthalmic delivery of pilocarpine for the pressure reducing effect which. It has shown significantly extended duration. [75] The hydrogel system has proved its efficient applications in nasal drug delivery system. The formulations containing polyvinylacetal diethylaminoacetate hydrogel with chlorpheniramine maleate were investigated for instillation into the nose and hydrogel formed on the mucous membrane provided controlled drug release. [76]

Patil et al. (2011, 2011, and 2012) have developed innovative hydrogel beads of lovastatin with gellan gum alone and in combination with chitosan by ionotropic gellation technique. The natural polymer based

beads were characterized and evaluated for various physicochemical properties. The drug release from ionically cross linked gellan beads was rapid and chitosan based beads showed considerably prolonged drug release. DSC and SEM results revealed that the drug is in the amorphous status and uniformly dispersed in the polymer matrix and the beads were found to be spherical in shape with smooth surface possessing inward dent and shrinkage and also give a hint of presence of minute pores. The authors also have developed prolonged release drug delivery system of stavudine by ionotropic gelation and polyelectrolyte complexation technique. Cross linking reinforced chitosan-gellan and chitosan-alginate complex beads were prepared by gelation of anionic gellan gum as primary polymer, with oppositely charged counter ions to form beads which were further complexed with chitosan as a polyelectrolyte. Effects of this polymer on release profile of drug were studied. The reaction of chitosan-gellan and chitosan-alginate complex dominates the formation of skin layer on the surface of beads. The final formulations were subjected to *in vitro* evaluation and several characterization studies. Batches with gellan gum and alginate alone followed Higuchi model and while chitosan-gellan and chitosan-alginate showed zero order release [77-79].

Biomedical Applications of hydrogels

Apart from pharmaceutical applications, hydrogels have vastly been utilized for versatile nature. The biomedical field stands next to pharmaceutical field in exploring the use of this system for various biomedical purposes ranging from diagnostic devices [80] to artificial muscles. [81] Hydrogel system is effectively used in the preparation of ophthalmic utensils such as contact lenses and intraocular lenses. Soft contact lenses made up of hydrogels possess excellent high oxygen permeability and also have ability to fold thus allows the surgeons to opt for much smaller surgical incisions. In surgical dressing, hydrogels are commonly applied as wound dressing materials as they are flexible, durable, non antigenic and are permeable to water vapors and metabolites [82]. Hydrogels can also be used in the treatment of cartilage defects, for this purpose a modified polysaccharide that occurs in cartilage has been used in formation of hydrogels. [83] Hydrogels are also used in the development of artificial muscles, smart hydrogels can function like human muscle tissue by transforming electrochemical stimuli into mechanical work. Methyl cellulose hydrogels have been employed to deliver allergens in skin testing. Hydrogels are often coated on the urinary catheter surfaces not only to provide smooth slippery surface but also to prevent bacterial colonization and improve biocompatibility. [84, 85] Hydrogels found to exhibit applications even as a wound healing materials. Honey hydrogels have been used for prompt wound healing by crosslinking the honey into a hydrogel matrix is more acceptable and peeled with a transparent system. [86] The hydrogel of gelatin and polyvinyl alcohol with blood coagulant reported to show better adhesive property than corresponding gel or ointment in controlling blood coagulation. [87] Hydrogels have also been designed for augmenting vocal cords and prevention of scar formation after surgery. [88] Hydrogels play an indispensable role in formation of layers on the inner surface of injured arteries to reduce thrombosis and initial thickening which can be prevented by inhibiting contact between blood and subendothelial tissue with a hydrogel layer. A unique swelling behavior of certain hydrogel system was rightly employed in stabilization of bone implants. [89] The potential applications of hydrogels have been explored in family planning sterilization. The fallopian tubes of the rabbits can be blocked by placing *in situ* gel forming hydrogels through transcervical catheterization and conception can be prevented. The structurally rigid and biocompatible hydrogels can be efficiently used in the tubular sterilization. Some prostaglandin analog hormones can be delivered in the form of hydrogel rods to mechanically dilate the cervix for the first trimester-induced abortion. [90, 91]

Miscellaneous applications of hydrogels

Hydrogel system can also be utilized in many other areas other than pharmaceutical and biomedical fields. In cosmetic field, silicon elastomer shells were filled with hydroxyl propyl cellulose polysaccharide gel and such shells were implanted in breast to accentuate them for aesthetic purpose. [92] Many hydrogels have been utilized for industrial applications as absorbents for effluents like methylene blue dye. [93] Other example of hydrogels is the absorption of dioxin by the DNA of Salmon milt hydrogel beads to prevent the health hazards like carcinogenicity, immunotoxicity or endocrine disruption caused by dioxin. Hydrogels are utilized to prevent the contamination of electrolysis products by making them to bridge metallic electrodes and

biological system. [94] The reversible swell and shrink nature of the smart hydrogels in the solution environment can be explored to prepare purification devices. [95]

CONCLUSIONS

Past few decades has witnessed a revolutionary advancement in the area of development of novel drug delivery system. With this advancement, many existing drug molecules have been revived of their therapeutic benefits. The introduction of hydrogel drug delivery systems has further strengthened the relationship between therapeutic need and drug delivery. After having an elaborative glance on the above review with no other second thought one can conclude that the hydrogels are 'smart; and 'intelligent' drug delivery devices which offered exciting applications. Due to the fascinating properties of stimuli responsiveness, hydrogels can be efficiently used as the effective drug delivery devices in some special cases. With their biocompatible and biodegradable nature, hydrogels have widely been used in the field of nanobiotechnology. Hydrogels are also having marvelous applications in the field of tissue engineering and biomedical as well. In the research field of novel hydrogel drug delivery, many aspects have been addressed properly to avail the right utility of this system for the betterment of mankind. Still there is urgent need in some other aspects of this system especially in the grafting of hydrogel polymers, understanding the critical swelling and relaxation behavior of the novel anionic and cationic polymers and characterization of this system to tailor their properties to fit in specific purposes. Further, the *in vitro*, *in vivo* correlation in the performance of the polymeric networks for improved therapeutic efficacy will determine their future success and open newer arenas in drug delivery.

REFERENCES

[1] Amin S, Rajabnezhad S, Kohli K. Hydrogels as potential drug delivery systems. Sci Res Essay. 2009; 11: 75-1183.

[2] Peppas NA, Bures P, Leobandung W, Ichikawa H. Hydrogels in pharmaceutical formulations. Eur J Pharm Biopharm. 2000; 50: 27-46.

[3] Stanty M, Plocova D, Etrych T, Kova M, Ulbrich K, Richova B. HPMA- hydrogels containing static drugs kinetics of the drug release and in-vivo efficacy, J Control Rel. 2002; 81: 101-111.

[4] Lowman AM, Peppas NA. Hydrogels. In: Encyclopedia of Controlled Drug Delivery, New York: Mathiowitz E ed., Wiley; 1991: 397-418.

[5] Peppas NA, Mikos AG. Preparation methods and structure of hydrogels, in: Hydrogels in medicine and pharmacy. Peppas NA. ed. Boka Raton FL: CRC Press; 1986: 1-27.

[6] Brannon-Peppas L, Herland, RS. (Eds). Preparation and characterization of crosslinked hydrophilic networks, in: Absorbent polymer technology. Elsevier, Amsterdam; 1990: 45-66.

[7] Kamath K, Park K. Biodegradable hydrogels in drug delivery. Adv Drug Deliv Rev. 1993: 85-108.

[8] Park K, Shalaby WSW, Park H. Biodegradable hydrogels for drug delivery, Technomic Lancaster; 1993.

[9] Jagur-Grodzinski. Polymeric gels and hydrogels for biomedical and pharmaceutical applications. Polym Adv Tech. 2010; 27-47.

[10] Pal K, BantHia AK, Majumda DK. Polymeric hydrogels: characterization and biomedical applications- A mini review. Design Monom Polym. 2009; 12: 197-220.

[11] Haffman AS. Hydrogels for biomedical applications. Adv Drug Deliv Rev. 2002; 54: 3-12.

[12] Rowley J, Madlambayan G, Faulkner J, Mooney DJ. Alginate hydrogels as synthetic extracellular matrix materials. Biomater. 1999; 20: 45-53.

[13] Liu Q, Hedberg EL, Liu Z, Bahulekar R, Meszlenyl R, Micos AG. Preparation of macroporous poly 2- Hydroxyethyl methacrylate hydrogels by enhanced phase separation. Biomater. 2000; 21: 2163-2169.

[14] Aroca AS, Ribelles JL, Pradas MM, Garayo AV, Anton JS. Characterization of polymethyl methacrylate coated with plasma polymerized poly 2-polyethylacrylate. Eur Polym J. 2007; 43: 4552-4564.

[15] McNeill ME, Graham NB. Properties controlling the diffusion and release of water soluble solutes from poly (ethyloxide) hydrogels 1. Polymer composition. J Biomater Sci Polym Ed. 1993; 4: 305-322.

[16] Jhan MS, Andrade JD. Water and hydrogels. J. Biomed Mater Res. 1973; 7: 509-522.

[17] Guenet JM. Themoreversible gelation of polymers and biopolymers, Academic Press, New York, 1992.
[18] Li L, Shan H, Yue CY, Lam YC, Tam KC, Hu X. Thermally induced association and dissociation of methyl cellulose in aqueous solutions. Langmuir. 2002; 18: 7291-7298.
[19] Zhiyuan P, Fangeng C. Synthesis and properties of temperature sensitive hydrogel based on hydroxyethyl cellulose. Int J Polym Mater. 2010; 59: 450-461.
[20] Bhattrai N, Ramay HR, Gunn J, Matsen FA, Zhang M. PEG-grafted chitosan as an injectable themosensitve hydrogel for sustained protein release. J Controll Rele. 2005; 103: 609-624.
[21] Zhag X, Wu D, Chu CC. Synthesis and characterization of partially biodegradable, temperature and pH sensitive Dex-MA/PNIPAAm hydrogels. Biomater. 2004; 5: 4719-4730.
[22] Shirakawa M, Yamotoya K, Nishinari K. Tailoring Xyloglucon properties using an enzyme. Food Hydrocoll 1998; 12: 25-28.
[23] Ruel-Gariepy E, Leroux JC. In situ forming hydrogels-review of temperature sensitive systems. Eur J Pharm Biopharm. 2004; 58: 409-426.
[24] Joly-Duhamel C, Hellio D, Djabouvor M. All gelatin network: 1. Biodiversity and physical chemistry. Langmuir 2002; 18: 7208-7217.
[25] Yang H, Kao WJ. Thermoresponsive gelatin/monomethoxy poly (ethylene glycol) - poly (D, L-lactide) hydrogels: formulation, characterization and antibacterial drug delivery. Pharm Res. 2006; 23: 203-214.
[26] Patil JS, Kamalapur MV, Marapur SC, Kadam DV. Ionotropic gelation and polyelectrolyte complexation: The novel techniques to design hydrogel particulate sustained, modulated drug delivery system: A review. Digest J Nanomater Biostruc. 2010; 5: 241-248.
[27] Yoshi E. Cytotoxic effects of acrylates and methacrylates: relationships of monomer structures and cytotoxicity. J Biomed Mater Res. 1997; 37: 517-524.
[28] Nedkov E, Tsvetkova S. Structure of poly (ethylene glycol) hydrogels obtained by gamma irradiation. Radiat Phys Chem. 1994; 44: 81-87.
[29] Peppas NA, Keys KB, Torres-Lugo M, Lowman AM. Poly (ethylene glycol) containing hydrogels in drug delivery. J Control Rel.1999; 62: 81-87.
[30] Allcock HA, Ambrosio AMA. Synthesis and characterization of pH sensitive poly (organophosphazene) hydrogels. Biomater. 1996; 17: 2295-2302.
[31] Stringer JL, Peppas NA. Diffusion of small molecular weight drugs in radiation crosslinked poly (ethylene oxide) hydrogels. J Control Rel. 1996; 42: 195-202.
[32] Akkas P, Sari M, Sen M, Guven O. The effect of external stimuli on bovine serum albumin absorption capacity of poly (acrylamide/maleic acid) hydrogels prepared by gamma rays. Radiat Phys Chem. 1999; 55: 171-721.
[33] Kabanov VA, Papisov IM. Formation of complexes between complimentary synthetic polymers and oligomers in dilute solutions. Vysokolmol Soedin 1979; 21: 243-281.
[34] Bekturov EA, Bimendina LA. Inerpolymer complexes. Adv Polym Sci. 1982; 45: 1-119.
[35] Esuchida E, Abe K. Interactions between macromolecules in solution and intermolecular complexes. Adv Polym Sci. 1982; 45: 1-119.
[36] Klier J, Peppas NA. Struture and swelling behavior of poly (ethylene glycol)/poly (methacrylic acid) complexes, in: L. Brannon, N.A., Peppas, R.S., Harland (Eds.). Absorbent Polymer Technology. Elsvier, Amsterdam. 1990; 147: 169.
[37] Bell CL, Peppas NA. Biomedical membranes from hydrogels and interpolymer complexes. Adv Polym Sci. 1995; 122: 125-175.
[38] Peppas NA. Physiologically responsive gels. J Bioact Compat Polym. 1991; 6: 241-246.
[39] Soppimath KS, Aminabhavi TM, Dave AM, Kumbar SG, Rudzinski WE. Stimulus-responsive "smart" Hydrogels as drug delivery systems. Drug Dev Ind Pharm. 2000; 28: 957-974.
[40] Ottenbrite RM, Park K, Okano T, Peppas NA. Biomedical applications of hydrogels, Handbook. New York: Springer; 2010.
[41] Bajpai AK, Shukla SK, Bhanu S, Kankane S. Responsive polymers in controlled drug delivery. Progr Polym Sci. 2008; 33: 1088-1118.
[42] Okuyama Y, Yoshida R, Sakai K, Okano T, Sakurai Y. Swelling controlled zero order and sigmoidal drug release from thermoresponsive poly (N-isopropylacrylamide-o-butyl methacrylate)

hydrogels. J Biomat Sci Polym. 1993; 4: 545-556.
[43] Ramanan RM, Chellamuthu P, Tang L, Nguyen KT. Development of a temperature sensitive composite hydrogels for drug delivery applications. Biotechnol Progr. 2006; 22: 118-125.
[44] Bae YH, Okano T, Kim SW. On-off thermo control of solute transport II. Solute release from thermosensitive hydrogels. Pharm Res 1991; 8: 624-628.
[45] Tanaka T. Collapse of gels and critical end point. Phys Rev Lett. 1978; 40: 820-823.
[46] Ramanathan SH, Block L. The use of chitosan gels as matrices for electrically modulated drug delivery. J Controll Rele. 2001; 70: 109-123.
[47] Jensen M, Hansen PB, Murdan S, Frokjaer S, Florence AT. Loading into electro stimulated release of peptides and proteins from chondroitin 4-sulphate hydrogels. Eur J Pharm Sci. 2002; 15: 139-148.
[48] Sawahata K, Hara M, Yasunaga H, Osada Y. Electrically controlled drug delivery system using polyelectrolyte gels, J. Controll Rele. 1990; 14: 253-262.
[49] Anseth KS, Metters AT, Bryant SJ, Martens PJ, Elisseeff JH, Bowman CN. In-situ forming degradable networks and their application in tissue engineering and drug delivery. J Controll Rele. 2002; 78:199-209.
[50] Matsuda T. Device directed therapeutic drug delivery systems. J Controll Rele. 2002; 78: 125-131.
[51] Obara K, Ishihara M, Ishizoka T, Fujita M, Ozeki Y, Maehera T and et. al. Photoshrinkable chitosan hydrogel containing fibroblast growth factor-2 stimulates wound healing in healing-impaired db/db mice. Biomater. 2003; 24: 3437-3444.
[52] Ghandehari H, Kopeckova P, Kopecek J. In-vitro degradation of pH sensitive hydrogels containing aromatic azo bonds. Biomater. 1997; 18: 861-872.
[53] Akala EO, Kopeckova P, Kopecek J. Novel pH sensitive hydrogels with adjustable swelling kinetics, Biomater. 1998; 19: 1037-1047.
[54] Gupta P, Vermani K, Garg S. Hydrogels: from controlled release to pH-responsive drug delivery. Drug Discov Today. 2002; 569-579.
[55] Murthy N, Thng YX, Schuck S, Xu MC, Frecher JMJ. A novel strategy for encapsulation and release of proteins: hydrogels and microgels with acid-labile acetyl cross linkers. J Am Chem Soc. 2002; 124: 12398-12399.
[56] Bhardwaj TR, Kanwar M, Lal R, Gupta A. Natural gums and modified natural gums as sustained release carriers. Drug Develop Ind Pharm. 2000; 26: 1025-1038.
[57] Guo JH, Skinner GW, Harcum WW, Barnum PE. Pharmaceutical applications of naturally occurring water soluble polymers. Pharm Sci Technol Today. 1998; 1: 254-261.
[58] Starodoubtsep SG, Khokhlov AR, Sokolov EL, Chu B. Evidernce for polyelectrolyte/ ionomer behavior in the collapse of poly-cationic gels. Macromolec. 1995; 28: 3930-3936.
[59] Lee KK, Cussler L, Marchetti M, McHugh MA. Pressure dependent phase transitions in hydrogels. Chem Eng Sci. 1990; 45: 766-767.
[60] Zhong X, Wang YX, Wang SC. Pressure dependence of the volume phase transition of temperature sensitive gels. Chem Eng Sci. 1996; 51: 3235-3239.
[61] Albin G, Horbett TA, Ratner BD. Glucose sensitive membranes for controlled delivery of insulin: insulin transport studies. J Controll Rele. 1985; 2:153-164.
[62] Ishihara K, Obayashi M, Shinohara I. Glucose induced permeation control of insulin through a complex membrane consisting of immobilized glucose oxidase and a poly-(amine). Polym J. 1984; 16: 625-631.
[63] Brownlee M, Cerami A. A glucose controlled insulin delivery system: a semisynthetic insulin bond to lectine. Sci 1979; 206: 1190-1191.
[64] Hisamitsu I, Kataoka K, Koyama Y, Okano Y, Sakurai Y. Glucose responsive gel from phenyl borate polymer and polyvinyl alcohol: prompt response at physiological pH through the interaction of borate with amino group in the gel. Pharm Res. 1997; 14: 289-293.
[65] Hovgaard L, Brondsted H. Dextran hydrogels for colon specific drug delivery, J Controll Rele. 1995; 36: 159-166.
[66] Suzuki Y, Tanihara M, Nishimura Y, Suzuki K, Kakimaru Y, Shimizu Y. A new drug delivery system with controlled release of antibiotics only in the presence of infection. J Biomed Mater Res. 1998; 42: 112-116.
[67] Mosbach K. Molecular imprinting. Trends Biochem Sci. 1994; 19: 9-14.

[68] Wang GQ, Kuroda K, Enoki T, Grosberj A, Masamune S, Oya T and et. al. Gel catalysts that switch on and off. Proc Natl Acad Sci USA 2000; 97: 9861-9864.
[69] Watanabe M, Akahoshi T, Tabata Y, Nakayama D. Molecular specific swelling change of hydrogels in accordance with the concentration of guest molecules. J Am Chem Soc. 1998; 120: 5577-5578.
[70] Na K, Bae YH. Self assembled hydrogel nanoparticles responsive to tumor extracellular pH from pullulan derivative/sulponamide conjugate: Characterization, aggregation and adriamycin release in-vitro. Pharm Res. 2004; 19: 681-688.
[71] Trookman N, Rizer R, Stephens TJ, Trancik R. Atopic dermatitis advantages of a novel hydrogel vehicle. J Am Acad Dermatol. 2007; 75: 730-735.
[72] Chang JY, Oh YK, Kong HS, Kim EU, Jang DD, Nam KT. Prolonged antifungal effects of clotrimazol containing mucoadhesive, thermo sensitive gels on vaginitis. J Controll Rele. 2002; 82: 39-50.
[73] Bugalassi S, Chetoni P, Panichi L, Boldrini E, Saettone MF. Xyloglucan as a novel vehicle for timolol: pharmacokinetics and pressure lowering activity in rabbits. J Ocul Pharmacol Th. 2000; 16: 497-509.
[74] Nanjawade BK, Manvi FV, Manjappa AS. In situ forming hydrogels for sustained ophthalmic drug delivery. J Controll Rele. 2007; 122: 119-134.
[75] Cohen S, Lobel E, Trevgoda A, Peled T. A novel in situ forming ophthalmic drug delivery system from alginates undergoing gelation in the eye. J Controll Rele. 1997; 44: 201-208.
[76] Aikawa K, Mitsutake A, Uda H, Tanaka S, Shimamura H, Aramaki Y and et. al. Drug release from pH responsive polyvinylacetal diethylaminoacetate hydrogel, and application to nasal delivery. Int J Pharm. 1998; 168:181-188.
[77] Patil JS, Babakkanavar AM, Shiralshetti SS, Marapur SC, Kamalapur MV. Formualtion, evaluation and characterization of novel gellan-chitosan hydrogel beads as controlled drug delivery system of antilipiemic drug. Indian Drugs 2011; 48: 35-40.
[78] Patil JS, Kamalapur MV, Marapur SC, Shiralshetti SS. Ionotropically Gelled novel hydrogel beads: Preparation, characterization and in vitro evaluation. Indian J Pharm Sci. 2011; 73(5): 504-09.
[79] Patil JS, Kamalapur MV, Marapur SC, Shiralshetti SS, Kadam DV. Ionotropically gelled chitosan-alginate complex hydrogel beads: preparation, characterization and in vitro evaluation. Indian J Pharm Edu Res. 2012; 46(3): 248-52.
[80] Hoffman AS. Applications of thermally reversible polymers and hydrogels in therapeutics and diagnostics. J Controll Rele. 1987; 6: 297-305.
[81] Suzuki M. Amphoteric Poly (vinyl alcohol) Hydrogel as a Material of Artificial Muscle. Koubunshi Ronbunshu 1989; 46: 603-605.
[82] Corkhill PH, Hamilton CJ, Tighe BJ. Synthetic hydrogels VI. Hydrogel composites as wound dressings and implant materials. Biomater. 1989; 10: 3-10.
[83] Fenglan X, Yubao L, Jiang WX. Preparation and characterization of nano hydroxyl apatite polyvinyl alcohol hydrogel biocomposite. J Mater Sci. 2004; 39: 5669-5672.
[84] Nigam SC, Sakoda A, Wang HY. Bioproduct recovery from unclarified broths and immobilized adsorbents. Biotechnol Progress 1988; 4: 166-172.
[85] Graiver D, Durall RL, Okada T. Surface morphology and friction coefficient of various types of Foley catheter. Biomater. 1993; 14: 465-469.
[86] Yusof N, Hafiza AHA, Zohdi RM, Bakar ZA, Development of honey hydrogel dressing for enhanced wound healing. Radiat Phys Chem. 2007; 76: 1767-1770.
[87] Mukherjee D, Banthia AK. Preparation of adrenocrome hydrogel patch, gel ointment and the comparison of their blood coagulating and wound healing capability. Mater Manuf Process. 2006; 21: 297-301.
[88] Kresa Z, Rems J, Wichterle O. Long-term results at patients with hydrogel implants in vocal cords. Arch Otholaryngol. 1973; 17: 360-365.
[89] Netti PA, Shelton JC, Revell PA, Pirie C, Smith S, Ambrosio L and et. al. Hydrogels as an interface between bone and an implant. Bonfield Biomater. 1993; 14: 1098-1104.
[90] Molin A, Brundin J. Cervical Dilatation before First Trimester Elective Abortion: A Comparison between Laminaria and a Newly Developed Hydrogel. Gynecol and obstet invest. 1992; 34: 12-14.

[91] Maubon AJ, Thurmond AS, LaurentA, Honiger JE, Scanlan RM, Rounet JP. Selective tubal sterilization in rabbits: experience with a hydrogel combined with a sclerosing agent. Radiol. 1994; 193: 721-723.
[92] Adam TST, Crook T, Cadier MAM. A late complication following the`insertion of hydrogel breast implants. J Plast Reconst Aesthet Surg. 2007; 60: 210-212.
[93] Paulino AT, Guilherma MR, Reis AV, Campese GM, Muniz EC, Nozaki J. Removal of methylene blue dye from an aqueous media using superabsorbent hydrogel supported on modified polyccharide. J Colloid Interf Sci. 2006; 301: 55-62.
[94] Kindler DD, Bergethon PR. Non-toxic and durable salt bridges using hydroxyethylmethacrylate hydrogels. J Appl Physiol. 1990; 69: 373-375.
[95] Marchetti M, Cussler EL. Hydrogels as ultrafiltration devices. Separat Purif Methods 1989; 18: 177-192.