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Review Article

PECTIN BASED FORMULATIONS FOR BIOMEDICAL APPLICATIONS: A REVIEW

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ABSTRACT

Pectin is known as a miracle polymer of natural origin because of its excellent biodegradable and biocompatible nature. Pectin is commercially extracted from different citrus products like apple, pomace, and oranges under mildly acidic conditions. Pectin has been mainly divided in to major group's namely high methoxyl pectin and low methoxyl pectin. Pectin is a high value functional food ingredient which is widely used as a gelling agent and stabilizer in food industries. Pectin has been widely investigated for targeted drug delivery and other potential biomedical applications. Pectin is known to be rapidly degraded by colonic microorganisms and thus makes it a potential carrier for colon targeted drug delivery. Pectin based formulations have shown tremendous promise as novel biomaterials for development of implantable and prosthetic devices. This paper reviews the recent research progress in development of pectin based formulations (polymer hydrogels, films, tablets, microspheres, nanoparticles and scaffolds) and their biomedical applications.

Keywords: Pectin, formulations, controlled drug delivery, biomaterials.

INTRODUCTION

Pectin is an excellent carbohydrate polymer derived from mainly natural resources and it is the structural component of plant cell wall. Pectin is one of the major constituents of citrus by products and has good gelling properties [1-2]. Chemically, pectin is poly α 1–4-galacturonic acids, with varying degree of methylation of carboxylic acid residues (Figure 1)[3]. Pectins with low degree of methylation forms gel in presence of multivalent ions whereas pectins with higher degree of methylation forms gel in acidic media with the addition of different sugars, e.g., sucrose or glucose[4]. Structurally pectin can be divided in three main regions, smooth (also called as linear regions), hairy and branched regions. The degree of esterification of glacaturonic acid residues of pectin is the most important parameter which affects the solubility of pectin and its gelling and film forming properties. The degree of esterification varies according to the origin of plant source, when and where the plant has been harvested and processing conditions such as storage, isolation, purification and extraction etc. [5]. Pectin has been used primarily in food industry as a gelling agent and is widely used for the production of jams and jellies, fruit juice, confectionary products and bakery fillings [6-7]. The other important application of pectin includes stabilization of acidified milk drinks and yoghurts[8]. Most of the pectin used by the food industry comes from citrus or apple peel from which it is extracted at low pH and high temperature and is primarily a homoglalacturonan[9-10]. Pectin has received significant attention as high fibre diet which is beneficial to health. It is widely accepted that a high fibre diet is crucial for good health and pectin is an important soluble fibre constituent of fruit and vegetables. It is a known fact that pectin can reduce cholesterol levels, serum glucose levels and may also have anticancer activities[11-12]. Pectin and pectin oligosaccharides have shown to induce apoptosis in human adenocarcinoma cells [13].

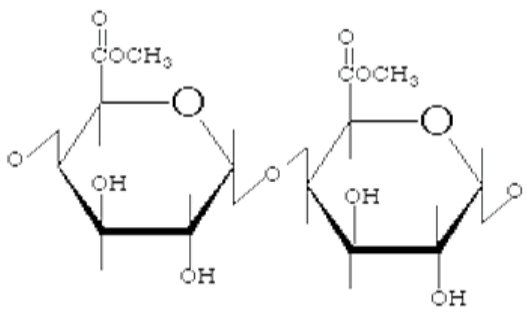

Figure 1: Chemical structure of pectin

Pectin and its formulations

From ancient times pectin has long and safe history of use in food applications. Pectin has the desirable stability under acidic conditions even at higher temperature[5] which makes them ideal candidate to be used in drug delivery system (DDS). Pectin has unique gel forming ability in presence of divalent cations which makes it an ideal carrier for delivering bioactive agents. Pectin forms an aggregate of macromolecules at low pH but at neutral pH the pectin aggregates tend to dissociate and forms an expanded network. It is a known fact that pectin is resistant to proteases and amylase which is active in upper gastrointestinal tract (GIT) and it is degraded by large number of microorganisms present in colon[14-16]. Recently tremendous interest has been shown by researchers to develop pectin based formulations for controlled delivery[17-18]. Pectin has long standing reputation of being non- toxic [19], their relatively low production cost[17] and high availability[20]. It was proposed that pectin can be used for delivering drugs orally, nasally, and vaginally[21-26] and it has been well accepted by patients[27]. Several pectin formulations (hydrogels, films, microspheres and nanoparticles) has been used so far targeting various proteins and drugs. There is already few excellent review articles published on pectin and its controlled delivery applications [5, 8, 10, 18, 28]. In this article we will review the recent research on various pectin based formulations being investigated for various biomedical applications.

Pectin based hydrogels

Hydrogels are three dimensional crosslinked polymeric materials which has ability to absorb and retain large amount of water. The soft and rubbery nature of hydrogels minimizes irritation to the surrounding tissues which makes them excellent candidate as an artificial skin and tissue engineering applications. The other important applications of hydrogels are as matrix for controlled drug delivery, soft contact lenses, protein separation and matrices for cell encapsulation [29-35]. Natural polymers have clear advantage over synthetic counterparts because of their biodegradable and biocompatible nature. However the mechanical strength of natural polymer formulation is less as compared to synthetic polymers which makes them vulnerable for various biomedical applications. Pectin has special place among available polysaccharides due to their biodegradable and non-toxic nature. But pectin formulations have some disadvantages such as premature drug release, low mechanical strength, low drug loading efficiency and less shear stability. As part of our research effort to develop pectin based formulations for controlled delivery we modified pectin by chemical (graft copolymerization and amidation) and physical (polymer blending) methods [36-42].

We developed pH sensitive polyacrylamide grafted pectin by simple graft copolymerization method which showed better gelling and film forming ability than pectin (Figure 2). We studied the effect of various reaction variables like initiator concentration, monomer concentration, reaction temperature and time on grafting [36]. The incorporation of amide group on the polysaccharide was confirmed by Fourier transform infrared (FTIR) spectroscopy and elemental (CHN) analysis. Furthermore differential scanning calorimetry (DSC) and x-ray diffraction (XRD) studies confirmed the formation of graft copolymer. The developed hydrogels were crosslinked with low amount of glutaraldehyde (GA) and the crosslinked hydrogel exhibited pH dependent swelling behaviour. Rheological studies showed that the graft copolymer hydrogel was more shear table than the natural polymer. We found that the developed pH sensitive hydrogels were fairly biocompatible in nature and showed higher cell viability (B-16 melanoma cells) even at the higher polymer concentration used [36, 38]. In a recent work we modified pectin by amidation reaction by using methanol as solvent (Figure 3). Pectin was chemically modified by ethanolamine and modified product was utilized for preparing hydrogel crosslinked with GA. The incorporation of amide groups on pectin lead to significant improvement in gelling as well as film forming ability. We demonstrated that the amidated pectin has excellent swelling nature and can effectively release salicylic acid at colonic pH for an extended period of time. The developed amidated pectin hydrogel was cytocompatible with B-16 melanoma cells [37, 39]. Ranjha et al [43] synthesized the pH sensitive pectin/acrylic acid hydrogels for verapamil release study. The hydrogels were synthesized by pectin and acrylic acid using N, N-methylenebisacrylamide (MBA) and benzoyl peroxide as initiator. The authors measured the equilibrium water content (EWC), diffusion coefficient (d), and volume fraction of polymer within the hydrogels (ϕ_2). They loaded verapamil as model drug in order to investigate its drug release from the hydrogel in USP phosphate buffer.

Recently pectin has been grafted with poly (N-isopropylacrylamide) and studied as a potential carrier for colon targeted drug delivery of theophylline[44]. They prepared the hydrogels by using ceric ammonium nitrate (CAN) as free radical initiator and MBA as crosslinker. They found that the hydrogel did not showed thermoresponsive nature. The authors suggested that optimum colon targeted vehicle provides less release at pH 5.5 and most drug release at pH 7.4. Yoshimura et al[45] prepared pectin based superabsorbent hydrogels crosslinked with three kind of crosslinkers, $CaCl_2$, ethylene glycol glycidyl ether (EGE), and glutaraldehyde (GA). The authors suggested that GA is the most suitable crosslinker among them because it is suitable to attain high water absorbency and hydrogels is sensitive to salt concentration. They suggested that the pectin hydrogel is an environmental friendly material because it exhibits excellent biodegradability.

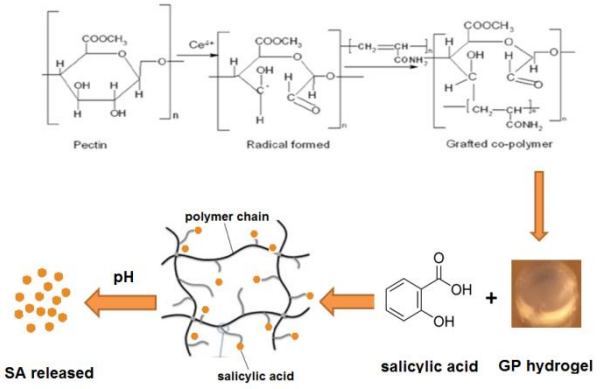

Figure 2: Mechanism of Graft copolymerization of pectin with polyacrylamide and controlled delivery of salicylic acid from the grafted pectin (GP) hydrogel

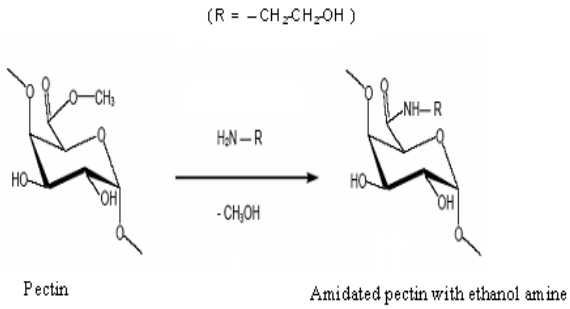

Figure 3: Reaction mechanism of amidation of pectin with ethanolamine

Pectin based films

Polymer blending has become an interesting technique to obtain materials with improved physicochemical properties. The binary blends of natural polysaccharides and their blends with synthetic polymers are promising systems for the development of new polymeric materials. These materials are interesting materials for the development of composite polymer films, sponges, hydrogels, ion exchangers and complexing agents, dressings, shells for encapsulating drugs, sutures, etc. The physiochemical and mechanical properties of such blends are determined by the type of bonds between the components, their compatibity and the features of forming supermolecular structure [46]. Nowadays interest has been shifted from synthetic polymers to bio based materials derived from agricultural or forestry resources because of increasing environmental concerns arising from non-biodegradable plastics and an awareness of limitation of petroleum based products [47]. Pectin is water soluble polymer with fairly good biodegradable nature which can be exploited for designing pectin films. The pectin derived films have shown potential applications in coating, encapsulating and thickening for food and pharmaceutical uses [47].

Hoagland et al[48] fabricated chitosan/pectin films with either glycerol or lactic acid as plasticizer to give clear laminated films with dynamic mechanical properties similar to those for pectin films alone. To prevent fungal growth on the laminated films they replaced glycerol with lactic acid without significant change in dynamic mechanical properties. They found out that storage and loss modulus of laminated films were significantly higher than respective moduli of chitosan films alone. Fishman et al[49] developed edible and biodegradable pectin/starch blend films plasticized with glycerol. They suggested that the developed films have the similar microsctructure and thermal dynamic mechanical properties to those obtained by casting from solution. They suggested that plasticized and pectin/starch films have a large glass transition at about -50°C indicating that these films are reasonably flexible at room temperature. They found that mechanical properties of the developed films were comparable to their previous research work[50]. Recently pectin and polylactic acid (PLA) film were fabricated for their intended applications in antimicrobial packaging [47]. They loaded model antimicrobial polypeptide, nisin, into the composite by diffusion method. The nisin loaded composite suppressed *Lactobacillus plantarum* growth, which was indicated by agar diffusion and liquid phase culture tests. Ghaffari et al [51] prepared free mixed film of pectin/chitosan/Eudragit RS intended for sigmoidal drug delivery. They added Eudragit RS which is a water insoluble polymer in the film in order to control the higher swelling of the film in aqueous media. They suggested that polyelectrolyte formation between pectin and chitosan resulted in a decrease in crystallinity and thermal stability caused by interaction between polyions. They loaded theophylline as a model drug in the film to measure permeability constant. This mixed film formulation showed potential for sigmoidal drug delivery with an initial controllable slow release followed by burst release immediately after change in pH.

Liu et al [52] developed composite films based on pectin, fish skin gelatin (FSG) and soybean flour protein (SFP). The resultant composite films showed an increase in stiffness and strength and a decrease in water solubility and water vapor transmission rate, in comparison to the film cast from pectin alone. The composite films inherited elastic nature of proteins, thus being more flexible than pectin films. They suggested that crosslinking the film with glutaraldehyde/methanol improves the Young's modulus and tensile strength. In another study Liu et al [53] developed pectin/poly (lactide-co-glycolide) (PLGA) composite matrices for the delivery of bioactive substances for tissue regeneration. They show that a new biodegradable matrix composed of hydrophobic PLGA; network entangled with another network of hydrophilic pectin was fabricated in presence of calcium chloride. They demonstrated that pectin enables the composite to carry signal molecules. Furthermore they suggested that PLGA imparts good mechanical properties in the composite and pectin helps in cell adhesion and proliferation as determined by osteoblast culture.

We recently developed novel polymer blend films based on pectin/polyvinyl pyrrolidone (PVP) and pectin/gelatin for their respective applications in controlled drug delivery and wound dressing applications[40-42]. Considering the film forming properties and biocompatible nature of PVP, it was blended with pectin and crosslinked with glutaraldehyde to improve its stability and mechanical properties (Figure 4). We demonstrated that pectin was completely miscible with PVP with a homogeneous amorphous phase. DSC studies on the blend membranes showed a single glass transition temperature which confirmed the miscible nature of blend and this was mainly due to intermolecular hydrogen bonding between hydroxyl group of pectin and carbonyl group of PVP. The XRD studies on the blend films showed that increase in PVP composition in the blend leads to increase in amorphous nature however it improves the mechanical properties of the blend film. The microscopic imaging (SEM) studies on blend film showed circular pores, which confirmed that drug (SA) diffuses out of the matrix and leaving behind the empty channels. In another recent study we investigated the potential of pectin/gelatin membranes as wound dressing material (Figure 5) [42]. We show that incorporation of gelatin in the membrane significantly improves the porous nature as well as improves the mechanical properties of the membrane. The water vapour transmission rate analysis showed the moisture retentive nature which can be exploited for wound care applications.

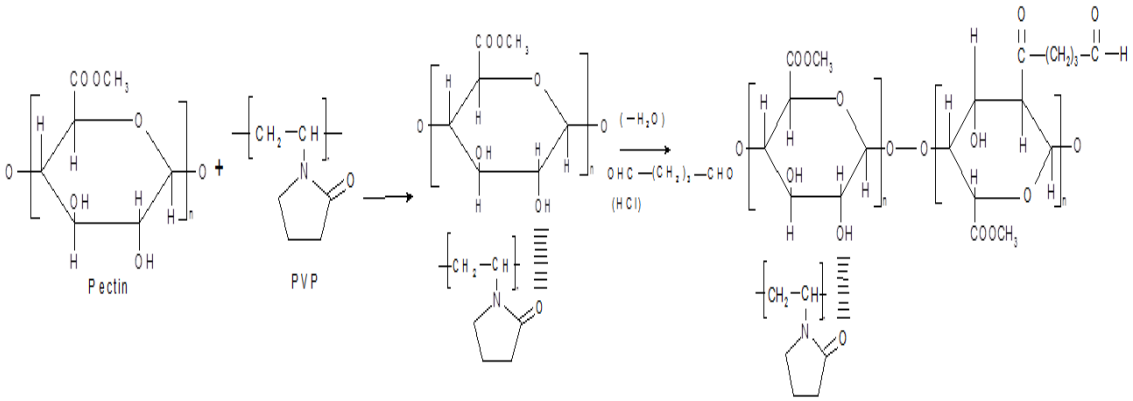

Figure 4: Reaction scheme for the preparation of pectin/PVP hydrogel crosslinked with glutaraldehyde (GA)

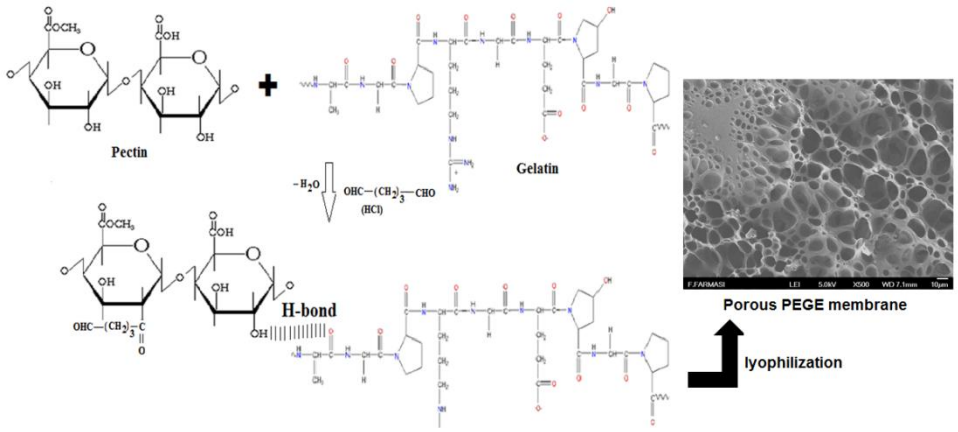

Figure 5: Mechanism of hydrogen bonding and crosslinking between pectin/gelatin membranes

Pectin based microparticles

Nowadays a great deal of research is focused on using microencapsulation technology for drug encapsulation and drug delivery [54]. It was reported earlier that microspheres can increase the life span of active constituents and can effectively control the release of bioactive agents [54]. The microspheres must fulfil certain criteria: (i) high encapsulation efficiency (ii) preservation of drug activity during encapsulation and storage (iii) easy administration to the target site (iv) Controlled release rate to achieve a therapeutic effect while minimizing side effects[55]. The microspheres have some benefits over other formulations that it can protect the encapsulated drug from the harsh conditions and a release profile for a desired period of time. Pectin based microspheres are gaining considerable importance because of their biodegradable nature and can be easily digested by the colonic microflora.

Recently Perera et al developed pectin-4-aminothiophinole (ATP) conjugate microparticles for colon specific drug delivery [56]. They prepared the microparticles by spray drying method and subsequent processing. They investigated the disintegration behaviour, particle size, drug load, release behaviour and impact on Caco-2 cells. They suggested that without colonic release inducers 34.4-fold more metronidazole is retarded in pectin-ATP microparticles within 6 hours compared with control particles. They postulated that the cell viability study did not show any significant difference between native and modified pectin, neither as a solution nor as microparticle suspension. In a recent work ondansetron was loaded into pectin

microspheres for intranasal administration [57]. The study was aimed to avoid hepatic first pass metabolism and enhanced residence time. They examined the effect of formulation and process variables on the characteristics of the microspheres prepared. They studied the effect of various experimental parameters such as drug to polymer concentration and liquid feed flow rate on particle size and entrapment efficiency by means of experimental factorial designs. Morphology analysis by them revealed smooth spherical surface of the microspheres and kinetic model showed that the drug release followed case II transport. They suggested that nasal delivery has increased the bioavailability as compared to oral delivery. Das et al recently studied the zinc-pectin-chitosan composite particles for drug delivery to the colon and investigated the role of chitosan in modifying in vitro and in vivo drug release [58]. They used resveratrol as model drug due to its potential activity on colonic diseases. They compared the in-vivo pharmacokinetic of zinc-pectinate particles with zinc-pectin-chitosan composite particles in rats. They found that formulation parameters significantly affect the drug release pattern from the formulation. Their pharmacokinetic evaluation showed the in vivo colon specific drug release from the zinc-pectin-chitosan composite particles only. Composite microparticle drug delivery systems based on chitosan, alginate and pectin with improve pH sensitivity for oral delivery of bovine serum albumin (BSA) was developed by Yu et al [59]. They show that microparticles were formed by tripolyphosphate crosslinking; electrostatic complexation of alginate and/or pectin, as well as ionotropic gelation with calcium ions, the microparticles exhibited improved pH sensitivity. They suggested that the microparticles had the potential for site specific protein delivery through oral administration.

The use of native degradable polysaccharides for development of drug delivery formulations is a greatest challenge because of their high aqueous solubility. This usually causes undesirable premature and localized release of the antibiotic[60]. Recently a multiparticulate system showing simultaneous biodegradability and pH dependent drug release was prepared based on chitosan, amidated pectin and calcium ions using triamcinolone as model drug[60]. They added hydroxypropyl methyl cellulose (HPMC) and cellulose pthalate (CAP) to aid the targeted action of the carbohydrates. The inclusion of these additives (HPMC and CAP) resulted in highest control over the drug release in all media. They suggested that although the enteric polymers can act as aid agents, the crucial condition to allow drug release in colon remains on the dependence of enzymatic degradation of polysaccharides by the microflora.

Pectin based tablets

In recent years a considerable research has been conducted to develop new oral dosage forms. Considering the quality of life, most of these efforts were concentrated on ease of medication. Among the various pharmaceutical formulations developed to improve the ease of administration, the matrix tablets are the most widely preferred. Pectin as a natural polymer has been widely investigated as a matrix tablet for colon specific drug delivery system. Wu et al[61] investigated the biphasic release of indomethacin from HPMC/pectin/calcium chloride matrix tablet and effect of variety of variables that to be is supposed to be encountered by the oral route. The authors added pectinase into the drug release medium and it triggered the matrix indomethacin release from the tablet. They found that the matrix tablet was stable under accelerated and long term testing conditions but stress testing indicated that release characteristics were affected by high relative humidity. Furthermore, pharmacokinetic study on dogs indicated that the in situ crosslinking of the matrix tablets could provide sufficient time delay, which may be related to more effective delivery of drugs to colon. Wei et al[62] investigated the sigmoidal release of indomethacin from pectin matrix tablets and studied the effect of in situ crosslinking by calcium cations. The authors suggested that calcium chloride incorporated in pectin matrix tablets functioned as retarding factor on drug release. Erosion correlated well with release in almost all pectin matrix tablets indicating erosion controlled mechanism.

Ugurlu et al [63] developed the nisin containing pectin/HPMC compression coated tablets and tested there in vitro behaviour for colonic delivery. Nisin is a 34-amino acid residue long, heat stable peptide belonging to the group. It is a lantibiotic with wide antimicrobial activity against Gram-positive bacteria. The authors suggested that the invention can be useful in treating colonic infectious diseases such as by *Clostridium difficile* and also by colonization of venomycin-resistant *enterococci*. They found that pectin alone is not sufficient to protect the nisin containing core tablets. It was found that the tablets maintained their integrity during the 6h dissolution test, approximately the colon arrival times. They suggested that polymer hydration effects the polymer degradation and found to be crucial for the enzymatic activity. They found that this matrix envelop was good for delivering nisin to colon. Recently Dev et al [64] developed the novel microbially triggered colon specific drug delivery system of 5-Fluorouracil and investigated its potential for colon cancer treatment. The authors evaluated the hardness, percent cumulative release at 5th hour. The release studies were conducted using change over media in presence of 4 % rat caecal contents. The optimized formulations were subjected to in vivo roentgenographic studies in New Zealand white rabbits to analyse the in vivo behaviour of the developed tablets. Roentgenographic studies corroborated the in vitro observations, thus providing the proof of concept. Pharmacokinetic studies revealed significant reduction in systemic exposure and cytotoxicity studies demonstrated enhanced cellular uptake of drug by the developed formulation. The authors suggested that the shelf life of the formulation was found to be 2.83 years. Furthermore they concluded that the established pectin based matrix tablet to be a promoting system to treat colon carcinoma.

Pectin based nanoparticles

Polymeric nanoparticles has received significant attention over the years and playing a pivotal role in delivering cancer chemotherapeutics and genetic therapy drugs. It was proposed that due to ultra-small volume, they can easily pass through tissue interstice and they can be absorbed by particular cells and can be removed by phagocytes [65]. The properties of polymer nanoparticles have to be optimized depending on the particular application. The method of preparation of nanoparticles plays an important role in order to achieve the desirable properties [66]. Polysaccharides have recently been investigated for preparation of nanoparticles because of their excellent physicochemical properties and biocompatible nature which is beneficial for biomedical use [67-69]. Recently thiolated pectin based nanoparticles has been developed and its potential for ocular drug delivery was investigated [70]. The thiolated pectin nanoparticles were prepared by ionotropic gelation method using magnesium chloride as ionic crosslinker and timolol maleate as the model drug. They showed that amount of crosslinker exert more pronounced effect on particle size of nanoparticles, while polymer concentration effects the drug entrapment. They showed that mucoadhesive nanoparticles can release drug for prolong period from the particles lodged in the cul-de-sac. Finally they suggested that thiolated pectin nanoparticle can provide significantly higher ex vivo corneal permeation of timolol maleate across excised goat cornea than the conventional aqueous solution. In a recent research Dutta et al developed oxaliplatin encapsulated in magnetic nanocarriers of pectin as a potential targeted delivery for cancer therapy [71]. Superparamagnetic iron oxide nanoparticles (SPIONs) and oxaliplatin (OHP) were in-situ encapsulated in pectin crosslinked with calcium ions forming 100-200 nm sized magnetically functionalized pectin nanocarriers. The microscopic imaging analysis (SEM and TEM) revealed the formation of spherical nanostructures. They confirmed the stability of aqueous dispersion of the nanocarriers by its high zeta potential. They achieved reasonably high encapsulation efficiency of OHP in these pectin based nanocarriers. They suggested that these nanocarriers showed sustained release of OHP in phosphate buffer solution (pH 5.5 and 7.4) and drug release profile satisfied a combination of diffusion and swelling controlled mechanism. Finally they concluded that fate of these MP-OHP nanocarriers as clinically relevant magnetic nanocarriers for targeted cancer therapy has to be confirmed by in vivo models. In another research they developed a novel probe sonication method for enhanced loading of 5-FU in SPION encapsulated pectin nanocarriers for magnetic targeted drug delivery[72]. They showed that probe sonication method significantly

improves the loading efficiency and corresponding loading content in the nanocarriers. They suggested that enhanced loading is attributed to increase in the rate of dissolution of 5-FU in pectin due to transmission of kHz order sonic waves which increases temperature and pressure in the medium due to formation and collapsing of cavitation bubbles [72]. Furthermore they showed that the nanocarriers with saturation magnetization exhibited pH responsive release of 5-FU in corresponding physiological fluids (pH 1.2, 5.5, 6.8 and 7.4).

Recently Jones et al [73] investigated the effect of polysaccharide charge on formation and properties of biopolymer nanoparticles created by heat treatment of β-lactoblobulin-pectin complexes. They prepared mixed solution of globular proteins (β-lactoblobulin) and anionic polysaccharides (high and low methoxyl pectin). They used micro-electrophoresis, dynamic light scattering, turbidity and atomic force microscopy measurements in order to determine the influence of protein-to-polysaccharide mass ratio, solution pH and heat treatment on biopolymer particle formation. They suggested that stability and size of the particles depended on type of pectin used, with high methoxy pectin giving smaller and stable particles than low methoxy pectin. They suggested that biopolymer particles formed appear to consist primarily of aggregated protein molecules, but they are probably complexed with pectin at pH values where there is sufficiently strong electrostatic attraction between protein and polysaccharide. Opanasopit et al [74] developed pectinate based micro/nanoparticles for gene delivery. The nanoparticles were developed by ionotropic gelation method. They evaluated the size and charge on pectin nanoparticles and DNA entrapment efficiency. The transfection of both calcium pectinate and Mg-pectinate nanoparticles yielded relatively low levels of green fluorescent protein expression and low cytotoxicity in Huh7 cells.

Kumar et al [75] investigated the suppression of agglomeration of ciprofloxacin-loaded human serum albumin (HSA)-pectin nanoparticles. The HAS-pectin nanoparticles loaded with ciprofloxacin was prepared by pH-coacervation method and various physicochemical properties were evaluated. The authors suggested that pectin may be used as pharmaceutical additive for the suppression of particle agglomeration in HSA nanoparticles and the effect may be attributed to the pectin segments present on the surface of nanoparticles. Recently Verma et al developed pectin nanoparticles and assessed its efficacy as a drug delivery vehicle for anticancer delivery of paclitaxel [76]. The authors suggested that the carboxylate, hydroxyl ions of pectin as well as amide group of L-Asparagine was also actively involved in the folding process forming the nanoparticles. The drug release pattern of the drug was biphasic, releasing 96 % of the drug in pH 5.8 as against 81 % in pH 7.4 over a period of month. Cheng et al [77] investigated the effect of pectin molecular weight and formulation pH on insulin loaded calcium pectinate nanoparticles. These nanoparticles were prepared as potential for colonic delivery system by ionotropic gelation with calcium ions. They suggested that milled pectin did not yield nanoparticles with significantly different mean diameter and insulin association efficiency compared to nanoparticles of unmilled pectin. The authors suggested that the subsequent release of associated insulin from the nanoparticles was depended on the extent of dilution of the nanoparticle dispersion and pH of the dissolution medium.

Pectin based scaffolds

Scaffolds are three dimensional porous biomaterials design to perform following functions: (i) promote cell biomaterial interactions, cell adhesion, and extra cellular matrix (ECM) deposition (ii) permit sufficient transport of gases, nutrients, and regulatory factors to allow cell survival, proliferation, and differentiation, (iii) provoke a minimal degree of inflammation or toxicity in vivo (iv) biodegrade at a controllable rate that approximates the rate of tissue regeneration under culture conditions of interest [78-79]. There has been ever-growing interest in tissue engineering techniques where biocompatible and biodegradable materials can be used as support matrices or as substrate for the delivery of bioactive species and cultured cells to targeted tissues and to promote three dimensional tissue reconstructions [80]. In a recent research Coimbra et al [81] prepared pectin/chitosan polyelectrolyte complex scaffolds for possible bone tissue engineering applications. The scaffolds were fabricated by freeze drying of the polyelectrolyte complexes formed between the two polysaccharides in an aqueous solution at pH 4.5. The weight loss studies on the scaffolds showed that it loses approximately half of its mass after a month of submersion in phosphate buffer solution at pH 7.4. The in vitro studies with human osteoblast cells showed that these adhere and proliferate on the surface of the scaffold, proving the biocomptability and non-cytotoxicity of the biomaterial. Munarin et al investigated pectin based injectable biomaterials for bone tissue engineering [82]. The authors suggested that pectin, modified with a RGD-containing oligopeptide or not can be used as an ECM alternative to immobilize cells for bone tissue regeneration. They confirmed that chemical grafting of the peptide containing the RGD sequence on pectin structure improve preosteoblasts adhesion, stimulating their metabolic activity and differentiation. Furthermore they showed that pectin and RGD-pectin microspheres incubated in the culture medium induced the deposition of calcium phosphates, thus behaving as biomimetic scaffold.

In another published work Lin et al [83] investigated the controlled release of pentoxifylline (PTX) from porous chitosan-pectin scaffolds. They proposed that tissue engineered scaffolds that can slowly release anti-inflammatory drugs can help reduce inflammatory reactions around implants. They observed that increase in pectin content in scaffolds increase wettability, less swelling and less capable of releasing PTX. The in vitro test showed the reduction of PTX release rates, PTX became more effective in inhibiting TNF-α and IL-6 production from activated macrophages.

Other Formulations

Recently Shaikh et al [84] developed crosslinked pectin based wafer matrices for gradual buccal delivery. The authors prepared three sets of drug loaded crosslinked pectin wafers by employing the model water soluble antihistamine, diphenhydramine and were compared with non-crosslinked wafers. The wafers were crosslinked with $CaCl_2$, $BaCl_2$ and $ZnSO_4$ pre or post lyophilization. They suggested that drug release performance was dependent on wafer production crosslinking sequence. The authors suggested that molecular mechanism simulation corroborated with the experimental data and established that Ba^{++} having largest atomic radii formed a number of ionic bridges producing wafers of high porosity and more influence on drug release. Luppi et al [85] developed chitosan/pectin based nasal inserts to improve bioavailability of antipsychotic drugs in the treatment of psychotic symptoms. They prepared chitosan/pectin polyelectrolyte complexes at pH 5 with different polycation/polyanion ratios and lyophilized in small inserts in presence of chlorpromazine hydrochloride. They suggested that the presence of increasing amount of pectin allows interaction with chlorpromazine hydrochloride inducing the formation of less hydratable inserts thus limiting drug release and permeation. The authors suggested that these nasal inserts are capable of achieving antipsychotic delivery in the nasal cavity.

In a recent research pectin based nanoemulsions loaded with a poorly water soluble drug itraconazole (ITZ) for pharmaceutical applications [86]. Nanoemulsions were prepared by simple homogenization to avoid high pressure conditions. They suggested that pectin with high degree of esterification offered good emulsion properties because of its high amount of hydrophobic molecules. It was found that addition of ITZ to the emulsion formulation was essential to obtain nanosized emulsions, resulting from molecular associations between the drug and pectin. Furthermore, they suggested that obtained nanoemulsions may be subsequently developed as self-emulsifying drug delivery system.

CONCLUSION AND FUTURE PROSPECTS

Pectin is one of extensively studied natural biodegradable polymer formulations for drug delivery, wound dressing and tissue engineering. Pectin has numerous benefits as formulation because it can be easily tailored in to hydrogels, films, scaffolds, microparticles, and nanoparticles. The highly hydrophilic nature of pectin is the major drawback for it to be used in pharmaceutical formulations. To overcome these shortcomings pectin formulations can be crosslinked with calcium ions, by blending with hydrophobic

polymers, by thickening the coating layer and inhibit the activity of protease or isolate them from the incorporated drugs by using a physical barrier. The chemical modification of pectin by using chemical and physical methods is believed to play crucial role in improving the inherent properties of the macromolecule. It was established earlier that pectin derivative carrying a higher charge density (either positive primary amine and negative carboxy groups) were able to penetrate deeply in tissues, thus prolonging the residual time to incorporate drugs and enhancing their penetration. Pectin has been used from ancient times in food applications but its application in controlled delivery is still infancy. As the research and development continues in pectin based formulations, we expect to see many innovative and exciting applications in near future.

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