

**Development of polyion-complex hydrogels as an alternative approach
for the production of bio-based polymers for food packaging
applications: a review**

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Abstract

Development of packaging materials from renewable resources has for a long time been desirable for sustainability reasons, but with recent explosions in prices of petroleum products, this now becomes also more economically viable. This paper shows how fundamental chemistry underlying three forms of hydrogels – physical hydrogels, chemical hydrogels, and interpenetrating polymer networks (IPN) – can be integrated to produce a new family of food packaging materials from biopolymers, illustrated here using gelatin and low-methoxyl pectin as examples. Application of this technique to create hydrogels from bio-based materials offers the potential for developing novel, biodegradable packaging applications, particularly for foods, that meet the ever-increasing demands for natural and environmentally compatible materials.

Introduction

For more than fifty years, plastic polymers have been the most practical, economical, and useful solution for packaging applications due to their low cost; ready availability; excellent optical, mechanical, and barrier properties; heat sealability; and resistance against water and grease. Despite these advantages, environmentalists have urged replacement of plastics with materials from renewable resources because plastic films are neither totally recyclable nor biodegradable (Siracusa, Rocculi, Romani & Dalla Rosa, 2008), and may cause serious environmental and waste disposal problems. Although high development costs and lack of available alternate products limited progress in this direction, recent explosions in prices of petroleum products have brought this problem to the forefront again, emphasizing the limited nature of the crude oil resources and providing compelling economic incentive for the exploration of renewable alternatives based on biomaterials. Indeed, these changes in the competition scene make it both imperative and profitable to focus research on renewable bio-materials, addressing development of new techniques and methods that take specific advantage of unique and individual features of readily-available biopolymers.

Rising to this challenge, several research groups as well as industrial companies worldwide are now developing new eco-friendly packaging solutions to exploit the 'ecological' advantages of biopolymers in applications such as food packaging. The past decade has seen rapid growth of new materials from renewable resources (Siracusa et al., 2008; Sorrentino, Gorrasi & Vittoria, 2007; Tharanathan, 2003; Weber, Haugaard, Festersen & Bertelsen, 2002; Petersen et al., 1999; Miller & Krochta, 1997;

[Guilbert, Gontard & Gorris, 1996](#)). Biopolymers directly extracted from biomass (e.g. proteins, polysaccharides, lipids) or from micro-organisms (e.g. polyhydroxyalkanoates), as well as some produced by classical chemical synthesis (e.g. polylactic acid), have been used to develop new structures for biomedical applications such as tissue engineering and organ regeneration ([Skotak, Leonov, Larsen, Noriega & Subramanian, 2008](#); [Jaklenec, Wan, Murray & Mathiowitz, 2008](#); [Lai, Lu, Chen, Tabata & Hsiue, 2006](#)) and for controlled drug delivery systems in the pharmaceutical industry ([Ghaffari, Navaee, Oskoui, Bayati & Rafiee-Tehrani, 2007](#); [Wei, Sun, Wu, Yin & Wu, 2006](#); [Ofori-Kwakye & Fell, 2003](#)). Development of food packaging applications from biopolymers has lagged behind medical materials due to high cost, low strength, and poor water resistance. Until recently, the most exploited routes to overcome these limiting factors involved blending natural and synthetic polymers together or incorporating inorganic fillers ([Mangiacapra, Gorrasi, Sorrentino & Vittoria, 2006](#)). As an alternative, hydrogels can also offer new opportunities for design of efficient biopolymer packaging materials with desirable properties.

Hydrogels are hydrophilic three-dimensional networks of polymer chains capable of imbibing large amounts of water, on the order of thousands of times their dry weight. Since the first synthetic hydrogels in 1960 ([Wichterle & Lim, 1960](#)), there has been a growing interest in hydrogels. Early research addressed cell encapsulation and later, controlled drug delivery. For more than a decade, both synthetic and natural hydrogels have been proposed as scaffolding materials for regenerating tissues and organs within the new ‘tissue engineering’ field. Hydrogels are frequently found in everyday products such as contact lens, capsules for oral ingestion,

coatings, membranes, slabs, micro- and nano-particles. Cast into films and dried, hydrogels now are also being tailored for biodegradable packaging materials for food, cosmetic and pharmaceutical products ([Langmaier, Mokrejs, Kolomaznik & Mladek, 2008](#)).

Dry hydrogels from biomacromolecules exhibit a number of desirable properties for packaging films, particularly biodegradability and the possibility to incorporate cells, bioactive compounds and drugs. Furthermore, due to the chemical properties of functional groups along the molecule backbone, hydrogels can be developed as ‘smart’ tailored devices able to respond to specific external stimuli (e.g. pH and the temperature of the surrounding medium) that act as triggers to modify over time the release rates of compounds loaded in them ([Qiu & Park, 2001](#)). Therefore, stimuli-sensitive hydrogels differ from inert hydrogels in that they can “sense” changes in environmental properties and respond by increasing or decreasing their degree of swelling and thus their hydration state. These capabilities, particularly the volume-changing behaviour, have attractive applications, especially for biomedical hydrogels generation. It is noteworthy that for non-ionic hydrogels, the extent of swelling only depends on the chemical compositions of the polymers, the pH changes having a negligible effect. On the contrary, when ionic pairs are used to fabricate hydrogels, the swelling depends not only on the chemical composition of the gel but also on the pH of the surrounding medium ([Lin & Metters, 2006](#)).

At the same time, the very hydratability that allows creation of hydrogels also poses some disadvantages that have delayed practical applications. When a dry hydrogel (e.g., a packaging film) begins to absorb

water, hydration at the most polar, hydrophilic groups will take place, leading to the swelling of the matrix. As the polar groups are hydrated, the network exposes hydrophobic groups, which also interact with water molecules. After these primary interactions, the network will imbibe additional water, due to the osmotic driving force of the network chains towards infinite dilution. The additional swelling water is assumed to fill the space between the network chains, and/or the center of larger pores, macropores or voids. An obstacle to this additional swelling is given by the covalent or physical crosslinks. Thus, the hydrogel will reach an equilibrium swelling level. As the network swells, if the network chains are degradable, the gel will begin to disintegrate and dissolve, at a rate depending on its composition (Hoffman, 2002). Consequently, early hydrogel films provided poor moisture resistance with reduced tensile strength and load-bearing capability.

These problems must be solved to develop hydrogel films with enhanced properties useful for a wide range of applications. We propose that this can be accomplished by creative utilization of the forces driving interactions between molecules (Hoffman, 2002; Hoare & Kohane, 2008). Hydration is particularly problematic in single-biopolymer films where water-polymer interactions compete effectively with polymer-polymer interactions. Polymer-polymer interactions can be strengthened by combining biopolymers with different structures and introducing predominantly charge interactions rather than hydrogen bonding. Using this approach, three forms of hydrogels may be created:

- ‘Physical hydrogels’ form from electrostatic interactions between oppositely-charged biopolymers. Exploiting the charge-distribution

properties of the multiple macromolecules provides opportunities for producing polyion complexes with improved performances compared to networks of individual polymers.

- ‘Chemical hydrogels’ are created by using crosslinkers to covalently bridge biopolymers at specific selected sites.
- ‘Interpenetrating polymer networks’ (IPN) are physical entanglements of polymers that allow formation of distinct networks, each with its own specific properties. The interpenetrating network may be simple, involving individual molecules, or complex, involving different types of hydrogels.

Additive effects from simultaneous exploitation of multiple hydrogel forms may generate unique and distinctive properties that offer great promise for use in packaging, particularly for foods.

This paper reviews important features of the different forms of hydrogels and the biopolymers comprising them and shows how physical and chemical hydrogels can be creatively integrated with interpenetrating networks to produce a new family of films for food packaging applications using gelatin and low-methoxyl pectin as examples of suitable biomacromolecules. Industrial utilization of hydrogels as well as future research directions are also discussed.

Fundamental chemistry underlying development of hydrogels

Physical and chemical hydrogels can be produced from either natural or synthetic polymers. Both forms have heterogeneous organization of independent domains ([Figure 1](#)), although they differ in the nature of molecular associations forming the network ([Bordi, Paradossi, Rinaldi &](#)

[Ruzicka, 2002](#)). Physical hydrogels are organized in heterogeneous clusters of distinct domains formed by molecular entanglements, free chain ends, and molecular ‘hairpin’, ‘kinks’ or ‘loops’ held together by weak hydrophobic associations, ionic interactions, or hydrogen bonding ([Hoffman, 2002](#)). Also called ‘reversible’ or ‘pseudo’ gels, physical hydrogels exhibit high water sensitivity (degrade and even disintegrate completely in water) and thermo-reversibility (melt to a polymer solution when exposed to heat).

Because of the strong electrostatic interactions involved, pH is by far the most important and most widely used factor for controlling the strength and direction of physical hydrogels. For example, as shown by [Mekhloufi, Sanchez, Renard, Guillemin and Hardy \(2005\)](#), above pH 4.9 β -lactoglobulin and gum acacia coexist in solution as separate polymers due to Coulombic repulsion between their negative charges. Between pH 4.9 and 4.2 patches of positive charge on β -lactoglobulin allow ionic associations to develop between the two polymers, and complex clusters of hydrogel form. Total charge decreases but enough charged sites remain for water binding and solubilization. However, at pH 4.04 where the net charge is zero, interaction with water is minimized while polymer interactions are maximized yielding dense, highly structured coacervate networks. Under these conditions, the water and mixed-polymer phases separate and films can be formed by casting or spreading the hydrogel.

A special subset of physical hydrogels is IPNs, physical blends of two or more different polymers that interact with each other merely by physical entanglement. The individual networks may be formed simultaneously or sequentially ([Kosmala, Henthorn & Brannon-Peppas, 2000](#)). Neither

physical (e.g. ionic) associations nor chemical linkages are supposed to contribute to IPN entanglement, although either or both networks may be crosslinked internally. When crosslinking occurs in a single polymer, semi-IPNs are formed. Crosslinking in both polymers yields full-IPNs. Sharing specific properties of each individual network results in new combinations with better mechanical properties than conventional hydrogels (e.g. IPNs are usually stiffer) ([Khademhosseini & Langer, 2007](#)). Therefore, IPNs can be viewed as a hydrogel network engineering approach that enables the achievement of specific performances otherwise unattainable through traditional ways. Several examples of IPNs (both full and semi, from synthetic or natural polymers) for different purposes have been reported using different combinations: gelatin/dextran IPNs as materials for degradable implants ([Kosmala et al., 2000](#)); pH-sensitive IPN hydrogels composed of two networks: the first one formed by crosslinking of a reactive polymer precursor (copolymer of N,N-dimethylacrylamide, acrylic acid, N-tert.butylacrylamide, and N-methacryloylglycylglycine p-nitrophenyl ester) with an aromatic azo group containing diamine; the second formed by radical crosslinking copolymerization of N-(2-hydroxypropyl)methacrylamide with N,O-dimethacryloylhydroxylamine ([Chivukula, Dušek, Wang, Dušková-Smrčková, Kopečková & Kopeček, 2006](#)); temperature-sensitive chitosan-poly(N-isopropylacrylamide) interpenetrated networks with enhanced loading capacity and controlled release properties ([Alvarez-Lorenzo, Concheiro, Dubovik, Grinberg, Burova & Grinberg, 2005](#)); pH switching on-off semi-IPN hydrogel based on crosslinked poly(acrylamide-co-acrylic acid) and linear polyallyamine ([Zhang, Wu, Li & Wang, 2005](#)); pH-sensitive gelatin–DNA semi-

interpenetrating polymer network hydrogel (Liu, Li, Ye, Sun, Zhu & Yao, 2004); gelatin-poly(methacrylic acid) interpenetrating polymeric network hydrogels as a pH-sensitive delivery system (Vishal Gupta, Satish & Shivakumar, 2007); tough films produced by creating morphologies having dual phase continuity in an interpenetrating ‘sponge’ morphology (LaCoste, Schaich, Zumbrennen & Yam, 2005).

In contrast to physical gels, chemical hydrogels (also called ‘irreversible’ or ‘permanent’ gels) are networks of polymer chains covalently linked at strategic connection sites (Figure 1). Most commonly, crosslinking is not spontaneous but is deliberately induced by reaction with small molecules such as aldehydes (Hoare et al., 2008) or radiation (e.g. electron beam exposure, gamma-radiation, or UV light) (Jo, Kang, Lee, Kwon & Byun, 2005; Terao et al., 2003). Uneven distribution of crosslinking within the gel leads to development of some zones in which typical ‘reversible’ features are still dominant and other zones with permanent properties arising from the crosslinked network. Chemical hydrogels neither disintegrate nor dissolve in aqueous solutions. Rather, they hydrate and swell until an equilibrium state is reached, which in turn strictly depends on the extent of the crosslinking. The swelling process is governed first by the water binding at the hydrophilic sites of the biomolecules, followed by the entrapment in the gel network of one or more hydration layers which form a ‘shell-like’ structure around the biomacromolecules.

Biopolymers for forming hydrogels

Formation of physical hydrogels from single biopolymers is a well-known phenomenon generating structure in foods ([Dickinson, 2006](#)), but single-polymer hydrogels tend to form weak films due to limited interchain interactions. In contrast, generating physical hydrogel films from combinations of polyelectrolyte biomolecules with opposite charges offers distinct advantages arising from the strength of the multiple intermolecular associations involved. The most promising biopolymer pairs for hydrogel formation are protein-polysaccharide associations. Mixing counter-charged biopolymers leads to formation of compound coacervates and polyion (polyelectrolyte) complexes ([Figure 1](#)), supermolecular structures that may precipitate or gel under specific conditions of ionic strength, biopolymer ratio, and pH.

The specific nature of the biopolymers critically influences the properties attainable from the complexes. Stiff and rigid linear polysaccharides such as pectin and xanthan gum, when mixed with proteins, tend to form complexes well-suited for producing gels in the form of sheets, membranes and coatings. In contrast, more globular and flexible polysaccharides such as acacia gum generate spherical structures (e.g. micro- and nano-capsules) that can encapsulate active compounds and be embedded in films. Similarly, high molecular weight-flexible proteins are the most suitable for the protein-polysaccharide pairs development, due to the capability of withstanding the changes in biopolymer conformation involved in different types of associations (i.e. electrostatic, hydrophobic, physical entanglements) ([Turgeon, Schmitt & Sanchez, 2007](#)). Moreover, working with proteins with many different functional groups along the

backbone together with hydrophobic regions may afford several routes to obtain hydrogels with tailored properties.

As shown in [Table 1](#), different protein-polysaccharide combinations have been recently reported for food packaging applications, each one with specific advantages and disadvantages. However, none of them envisaged the use of the gelatin-pectin pair with the declared aim of exploiting their charge and functional properties to produce a complex hydrogel for forming films with enhanced performance.

Of the proteins noted here, gelatin is particularly attractive for forming hydrogel packaging because it is relatively inexpensive and biodegradable, and its structure enables multiple combinations of molecular interactions. Indeed, gelatin modifications can take place in correspondence of its amino, carboxyl, and hydroxyl groups, although in most case gelatin is modified via its lysine and hydroxylysine amino acids as well as the amino groups of the N-terminal amino acid. These can be modified in the neutral to slightly alkaline range without causing hydrolyzation and degradation of gelatine. The chlorides and anhydrides of carboxylic acids, sulfonyl chlorides, isocyanates, and epoxides are normally used for the reaction. The property profile of gelatin can be strongly affected through such modifications. For example, modification of lysine with succinic acid anhydride leads to a gelatin characterized by improved swelling in water. The reaction between hydrolyzed gelatin and long-chain fatty acid chlorides yields biodegradable, non-toxic and non-irritating detergents for cosmetics applications. Finally, modifications using poly-functional compounds (e.g. dialdehydes, poly-epoxides and poly-isocyanates) allow obtaining products able to withstand boiling water ([Schrieber & Gareis, 2007](#)). Hence gelatin offers many

possibilities to generate hydrogel films with tailored performances, excellent functional properties, and greater flexibility.

Gelatin has a long history of productive applications in food/beverages (as gelling, stabilizing, binding, health-promoting, clarifying agent), pharmaceuticals (as hard shell capsules), and photographic materials, and it is increasingly being used to synthesize microspheres to carry pharmaceuticals, bioadhesives for wound dressing, scaffolds for tissue engineering, artificial skin, and structural biomaterials such as osteosynthetic devices (Schrieber et al., 2007). Gelatin-based packaging films have been proposed as an alternative to many synthetic plastics (Braumer, Fischer & Koepff, 1989). Incorporating other synthetic or natural molecules such as polyvinyl alcohol, acrylates, chitosan, cellulose fibres, overcomes some problems with mechanical weakness of films from gelatin alone, but some limitations from high water solubility still remain.

Among polysaccharides, pectin appears to be a particularly promising biomacromolecule for a wide range of present and future industrial hydrogel applications (Liu, Fishman, Kost & Hicks, 2003; Bédié, Turgeon & Makhlouf, 2008). Pectin has long been established as a multifunctional molecule in the food industry where its gelling capabilities are utilized extensively (Mesbahi, Jamalain & Farahnaky, 2005). However, over the past decade, increasing attention has been focused on pharmaceutical and biomedical applications of pectin hydrogels for their wide range of functional properties and their close similarity to the structure of polysaccharides found in the extracellular matrices of mammals (Sriamornsak & Nunthanid, 1998; Liu et al., 2003; Liu, Fishman, Hicks & Kende, 2005; Liu et al., 2004). Within this subfield, use of pectin for food

packaging remains a new development reported by Fishman's group (Fishman & Coffin, 1998; Fishman et al., 2000; Fishman, Coffin, Onwulata & Konstance, 2004; Fishman, Coffin, Onwulata & Willett, 2006) and a few other pioneers (Jo et al., 2005; Alves et al., 2008).

In processing, ionotropic gelation and gel coating have been widely exploited for fabricating pectin hydrogels, especially for pharmaceutical applications (Sriamornsak, 2003). For food packaging, films obtained by casting and extrusion are most common because the technology is readily available at low cost (Fishman et al., 2006).

Electrostatic associations between pectin and gelatin have been already investigated by Gilsean, Richardson and Morris (2003), who established the feasibility of fabricating gelatin-pectin films. However, critical limitations in film strength, barrier properties, and moisture sensitivity still remain. This paper will show how hydrogels from biomaterials such as pectin and gelatin can be engineered to overcome these detriments for food packaging applications. Features of gelatin and pectin structures and chemistries important to generation of hydrogels and packaging films are outlined below. Extending similar considerations to other polysaccharides and proteins will facilitate identification of other candidate polymer pairs with significant potential for generating bio-based food packaging with high functionality.

Gelatin

Gelatin is a protein obtained by physical, chemical or biochemical denaturation and hydrolysis of collagen, the basic unit of collagenous tissues (Schrieber et al., 2007). Collagen consists of three polypeptide chains

supercoiled into right-handed triple-helix (tertiary structure) which form rod-like units of approximately 300 nm long and 1.5 nm in diameter. Collagen units are able to pack together side-by-side, being displaced roughly one-fourth the length of a single molecule (quaternary structure), as shown in [Figure 2](#). This self-assembly structure is stabilized by aldol crosslinks between two lysine or hydroxylysine aldehyde derivatives ([Tanzer, 1973](#)), which account for the high stiffness as well as the insolubility of collagen.

During the denaturation-hydrolysis process, collagen triple-helix organization is hydrolyzed at the sites where covalent crosslinks join the three peptides. The resulting product (gelatin) is a mix of smaller random coiled chains with a range of molecular weights; these chains form an aqueous solution when heated to approximately 40°C, then transform to a physical hydrogel on cooling through a sol-gel transition. Hydrogel formation is accompanied by a disorder-order rearrangement in which gelatin chains partially recover the original triple-helix structure of collagen, leading to what is called 'renatured' or 'structural' gelatin. The resulting three dimensional network exhibits amorphous main regions of randomly-coiled gelatin chains interconnected with domains of spatially ordered molecules (called 'microcrystallites') stabilized by hydrogen bonds between NH of glycine and C=O of proline. A highly ordered hydration shell with water bridges linking two groups on the same chain or on two different chains is critically important for stabilizing the molecular conformation as well as the interactions between triple helices ([Brodsky & Ramshaw, 1997](#)). This structural and functional role of water in gelatin gels, especially in

relation to the junction zones between different gelatin chains, must be emphasized.

From a molecular point of view, the amino acid sequence of gelatin exhibits a repeating pattern (Gly-Pro-Hyp) characterized by the presence of glycine every third residue, with the imino acids proline and hydroxyproline accounting for about 10% of the residues ([Brodsky et al., 1997](#)). Triplets containing hydrophobic, charged, and reactive groups mediate interactions between gelatin and other molecules. In particular, the presence of both basic and acidic groups along the backbone makes gelatin a polyampholyte able to complex both negatively and positively-charged polyelectrolytes at below and above its isoelectric point (pI), respectively. The simultaneous presence of hydrophilic and hydrophobic regions further enhances the multifunctionality of gelatin, and is an important feature for generating strong packaging with hydrogels ([Schrieber et al., 2007](#)).

Properties of gelatin-based hydrogels are strongly affected by intrinsic factors of the raw material, which in turn will influence the fabrication process and the characteristics of the final product (e.g. films/coatings). Particularly important are the origin, the conditioning process, and the Bloom value, whose influence has been extensively reviewed in two recent publications ([Gómez-Guillén, Pérez-Mateos, Gómez-Estaca, López-Caballero, Giménez & Montero, 2009](#); [Karim & Bhat, 2009](#)).

The film-forming ability of gelatin is well established. In general, gelatin films and coatings have reasonable strength and clarity but, being highly sensitive to moisture, are poor water vapor barriers ([Gómez-Guillén et al., 2009](#)). As food packaging, gelatin films are mainly deposited as coatings to extend the shelf-life of perishable foods, especially protecting

them from oxygen, light, and moisture exchange. Gelatin-based coatings have been used effectively on meats, poultry and seafoods ([Gennadios, Hanna & Kurth, 1997](#)) as well as on refrigerated and frozen foods ([Villegas, O'connor, Kerry & Buckley, 1999](#)).

Properties of gelatin films depend on the raw material used in the process ([Karim et al., 2009](#)), and thus vary considerably with the source and type of gelatin. Although appreciable differences have been reported among fish gelatins, especially between cold-water and warm-water fish gelatins, the major difference is between mammalian and fish gelatins. Mammalian gelatins yield stronger films, whereas fish gelatins generate more deformable films. Fish gelatins exhibit about 5-10°C lower gelling and melting temperatures but higher viscosities ([Leuenberger, 1991](#)). These differences are a consequence of lower levels of amino acids proline (pro) and hydroxyproline (hyp) in fish gelatins (~20%) compared to pigskin and bovine gelatins (~30%) ([Karim et al., 2009](#)). Proline and hydroxyl proline are also important factors in thermal stability of gelatin due to steric restrictions imposed by their pyrrolidine rings plus hydrogen bonds formed between these and other amino acids residues ([Sikorski, Scott & Buisson, 1984](#); [Te Nijenhuis, 1997](#)). With low pro/hyp, fish gelatins have limited thermostability at temperatures above 30°C, so they cannot be used in high temperature applications such as packaging films; they are, nevertheless, suitable for applications requiring melting points at room temperature or below (e.g. as binding agents in food formulations). A further hurdle in use of fish gelatins with food is the possibility of allergenic reactions with any gelatin leached into package contents ([Olsen et al., 2003](#)). On the positive side, fish gelatins contain more hydrophobic amino acids so their films

show significantly lower water vapor permeability (WVP) than films produced from mammalian gelatins ([Avena-Bustillos, 2006](#)).

In view of these considerations, the selection of the most appropriate gelatin appears to be a nodal point in the fabrication of gelatin-based hydrogels intended as starting matrices for the manufacture of food packaging films and/or coatings.

Pectin

Pectin, a linear polysaccharide composed of D-galacturonic acid units linked by α -1,4-glycosidic bonds, is extracted from citrus peels and apple pomace by hot dilute mineral acid at pH 1.5 – 3.5. Short hydrolysis times yield pectinic acids and high-methoxyl pectins while extended acid treatment de-esterifies the methyl esters to pectic acids and generates low-methoxyl pectins. Commercial pectins are thus marketed on the basis of their degree of esterification (DE), defined as the ratio of esterified galacturonic acid groups to total galacturonic acid groups ([Sriamornsak, 2003](#)). High methoxyl pectins (HM) have over 50% of their carboxyl groups esterified (i.e., DE > 50, usually > 69), whereas low methoxyl pectins (LM) have a DE < 50, often < 20.

Low methoxyl pectins provide the charge interactions required for hydrogels. LM pectins are linear polyanions (polycarboxylates) of about 300 to 1000 saccharide units, with polymer molecular weights ranging from 50,000 to 150,000 Da. Dissolved LM pectins do not gel because ionized carboxylate groups ($-\text{COO}^-$) along the molecule repel each other. In addition, each pectin chain is strongly hydrated, particularly around the carboxylate groups. However, in the presence of divalent cations (usually

calcium), ionic interactions between the divalent cation and carboxylate groups in the pectin chains promote the formation of junction zones between molecules in close proximity. Crosslinking and gel strength increase directly with calcium concentration and inversely with DE.

Galacturonate rings in the pectin chain are themselves rigid and lacking in intrinsic flexibility. Thus, pectate molecules pack as corrugated sheets in an ordered structure with a limited physical entanglement (Axelos & Thibault, 1991). This structure, combined with the pattern of ionic crosslinking, generates gels in the well known 'egg-box' model also postulated for alginates (Grant, Morris, Rees, Smith & Thom, 1973) and is responsible for the normally rigid and brittle texture of low methoxyl pectin gels.

Factors other than calcium concentration influence gelation of pectins. Pectin structure (molecular weight, distribution of carboxyl and other functional groups along the molecule backbone) is critical: a minimum of seven consecutive carboxyl groups is necessary to ensure gel stability via ionic calcium linkages (Powell, Morris, Gidley & Rees, 1982); gelation is suppressed by addition of rhamnose and acetyl groups, but enhanced by partial amidation; de-esterified pectins can be gelled by monovalent salts (Yoo, Fishman, Savary & Hotchkiss, 2003). Exogenous conditions of the solution such as pH, temperature, and ionic strength are also important (Powell et al., 1982; BeMiller, 1986). Gel formation normally occurs only at pH 2.8 to 4.0. Below this pH range, pectins hydrolyze, and above it repulsive negative charges prevent associations between chains. Low temperature (4-25 °C) also promotes gel formation.

With regard to food packaging applications, pectin gels have been used to produce thin films coated onto meat (Kang, Jo, Kwon, Kim, Chung & Byun, 2007), fresh fruit (Maftoonazad, Ramaswamy, Moalemiyan & Kushalappa, 2007), bakery products (Baeva & Panchev, 2005) and fried potatoes (Khalil, 1999), with the aim of improving the sensory attributes and extending shelf life. There also is increasing interest in the development and manufacturing of pectin-based food packaging materials. Because of the well-known rigidity and brittleness of pectin films, pectins have usually been combined with other polymers of either natural (Fishman, Coffin, Unruh & Ly, 1996; Coffin & Fishman, 1993; Coffin & Fishman, 1994) or synthetic origin (Fishman et al., 1998), to generate packaging films with greater flexibility and tensile properties similar to those of plastic films. However, even these complex films exhibited high water sensitivity that, together with the poor processing endurance, are the major obstacles limiting the expansion of pectin film applications according to Liu, Liu, Fishman & Hicks (2007). To overcome these limitations, these authors proposed blending pectin with two different proteins -- fish skin gelatin and soybean flour protein – to improve both mechanical strength and flexibility (Liu et al., 2007). Even with this modification, however, films disintegrated in high moisture environments. Only crosslinking of amino groups with glutaraldehyde reduced water solubility of the films.

Pectin films have been proposed for several potential industrial uses where either water binding is not a problem or the water-binding can provide distinct advantages. Examples are water soluble pouches for detergents and insecticides, flushable liners and bags, medical delivery systems, edible bags for soup and noodle ingredients (Fishman et al, 2000),

antimicrobial film with potential application as packaging material for post-harvest crop protection (Elsabee, Abdou, Nagy & Eweis, 2008). Although materials manufactured only from pectin exhibit established limiting factors, such food processing industry waste is a very good candidate for generating hydrogels in combination with other biopolymers to produce eco-friendly structures with improved properties. It is firstly due to its chemical structure, namely the presence of carboxyl groups carrying a negative charge at $\text{pH} > \text{pK}_a$ which enable exploiting electrostatic interactions with positive charged counterparts. Secondly, secondary interactions with other polymers (e.g. hydrogen bonds) may be promoted by the simultaneous presence of a high amount of $-\text{OH}$ groups along the backbone. These same groups would also play a pivotal role in enhancing the gas barrier properties of pectin-based packaging materials, similarly to what happen with other polymer structures with a high $-\text{OH}$ density, like polyvinyl alcohol (PVOH). Finally, the regular and ordered structure of pectin molecules can contribute to improve the mechanical strength of the derived materials.

Development of integrated hydrogel films from gelatin and pectin

1. Formation of Physical Hydrogels

Given the multifunctionality of pectin and gelatin described in the previous section, various combinations of electrostatic interactions can be exploited to create mixed gelatin-pectin physical hydrogels as starting materials for packaging films. The structure attained is illustrated in [Figure 3](#). Both type A and type B gelatins of different origin as well as pectins of different DE and degree of amidation (DA) can be used ([Table 2](#)). The final hydrogel characteristics will depend on the specific combinations of

materials and conditions. Appropriate operative conditions for the fabrication of physical hydrogels must be determined for each gelatin-pectin combination because each system will behave in its own specific fashion; a single standard procedure can not be established to cover all hydrogel variants.

After testing a number of combinations, we found that combining LM pectin (DE = 7) from citrus with type A pigskin gelatin (250 Bloom) is a promising candidate for the fabrication of hydrogels suitable for producing films and coatings for food packaging applications (Farris, Schaich Liu, Cooke, Yam. Gelatin-pectin composite films from polyion complex hydrogels. *Journal of Agriculture and Food Chemistry*, submitted).

The first requirement for promoting ionic interactions between these molecules is control of pH in the reaction mixture. While the pI of gelatin is ≈ 8.5 , the operating pH must be maintained below pH 4.3 (the pK for glutamic COOH) for gelatin to have full positive charges and behave as a polyelectrolyte. However, protonation of pectin at pH values below its pK (pH 3.5) hinders electrostatic associations with gelatin and promotes pectin self-association via hydrogen bonding between COOHs, with formation of gel clusters. If the pH is lowered below ~ 2.5 , both macromolecules are hydrolyzed. Thus, the optimum pH value of 4.2 found for formation of gelatin-pectin hydrogels is high enough to ensure full ionization of the pectin COOH's, maximizing ionic linkage sites, but low enough that ionization of gelatin acid groups is incomplete, thus limiting repulsions. Not surprisingly, this pH is also close to that of both gelatin and pectin water solutions.

The second important parameter controlling hydrogel formation is charge balance, which is manipulated by the gelatin-pectin ratio in the mixed dispersion. Combining gelatin and pectin in different stoichiometries alters the positive/negative charge balance and hence the extent of associations between these two polyions. Manipulation of the associations requires knowledge of the net charge (millimoles of charge per gram of sample) distributed in the two molecules, which can be easily determined by titration. Formulations prepared at charge balance (lowest gelatin solubility) will display flocks in the solution. To obtain homogeneous solutions, either gelatin or pectin should dominate to provide the surplus electrical charge necessary for solubilization (Gilsenan et al., 2003). However, when the molar excess is too great, gelatin-gelatin or pectin-pectin associations compete and hydrogel films assume the properties of the concentrated component. High gelatin concentrations yield elastic and stretchable films, whereas high pectin films exhibit stiffer and more brittle layers.

Combining 13% gelatin and 1% pectin (w/w) in solution generates a physical polyion complex composed of a main gelatin network augmented by additional associations through ionic interactions with pectin, with no evidence of either large domains from self-aggregations or precipitation upon complex formation (Farris, Schaich Liu, Cooke, Yam. *Gelatin-pectin composite films from polyion complex hydrogels. Journal of Agriculture and Food Chemistry*, submitted). The ionic interactions occurred between the high distribution of negatively charged carboxyl groups on the pectin backbone and the positive amino groups of arginine, lysine and, to a lesser extent, histidine of gelatin (Figure 3). The thermodynamic driving force for the spontaneous gelatin-pectin associations appears to be enthalpic (negative

ΔH value), with the favourable entropic contribution ($-T\Delta S$) playing only a minor role (Ou & Muthukumar, 2006).

Results from our preliminary studies seem corroborate the hypothesis according to which physical hydrogels obtained from ionic interactions between gelatin and LM pectin have enhanced performance and favourable mechanical and water-sensitivity properties, especially when compared to the films composed of either gelatin or pectin alone. Accordingly, we believe that exploiting electrostatic interactions between counter-charged biopolymers represents a first step towards generating biopolymer films with improved performance (Farris, Schaich Liu, Cooke, Yam. Gelatin-pectin composite films from polyion complex hydrogels. *Journal of Agriculture and Food Chemistry*, submitted). Although gelatin-pectin composite films from physical hydrogels demonstrated improved overall performance relative to single polymer films, they still showed the same water sensitivity limitations noted by Liu et al. (2007). High water binding negatively under humid conditions leading markedly reduces stiffness and toughness, and the films disintegrate in water over time. Such drawbacks must be overcome before hydrogels produced from renewable resources can be fully developed and substituted for petroleum-derived plastics in commercial products.

Chemical Hydrogel

One approach to overcoming strength and water-binding limitations of physical hydrogels has been to synthesize permanent hydrogels by crosslinking one polymer chain (of both synthetic and natural origin) to another. Although this result can be accomplished by different ways, as

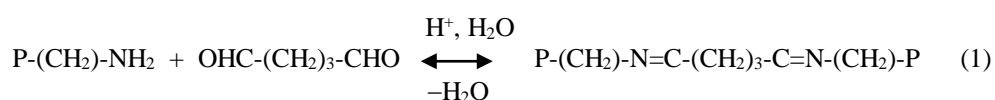
previously mentioned, small molecules with functional groups that covalently bind reactive groups are the most widely used crosslinking technique with natural biopolymers. This is because the crosslinkers can be specifically tailored to available sites on the polymers and the reaction can be controlled with relative ease. In the gelatin-pectin system, crosslinking of pectin is limited to divalent cations (Ca^{2+} in particular). However, the amino acid composition of gelatin provides multifunctional crosslinking options in side chains amino, carboxyl, and hydroxyl groups that react with a wide variety of established crosslinkers such as formaldehyde, glutaraldehyde, polyepoxy compounds (e.g. ethylene glycol diglycidyl ether), tannic acid, dimethyl suberimidate, carbodiimides and acyl azide (Schrieber et al., 2007). Unfortunately, most of these reagents are cytotoxic. Thus, in the last decade increasing effort has been focused on identifying naturally-occurring crosslinkers with low toxicity for use in applications where health hazardous substances is forbidden by law. Genepin (diphenylphosphorylazide, 1-ethyl-3-(3-dimethylaminopropyl carbodiimide hydrochloride), a natural crosslinker commonly employed as a tissue fixative, has been applied successfully for crosslinking gelatin (Sung, Liang, Chen, Huang & Liang, 2001) while glyceraldehyde shows little to no ability to fix collagenous materials (Kosmala et al, 2000).

Although bifunctional glutaraldehyde [$\text{OHC}-(\text{CH}_2)_3\text{-CHO}$] is not approved for food contact applications, it has demonstrated exceptional efficacy in collagen stabilization, while it is inexpensive and offers the possibility to reduce its toxicity by using very low concentrations (Bigi, Cojazzi, Panzavolta, Rubini & Roveri, 2001). Thus, we tested it at low levels (0.3%^{w/w}) to assess how chemical crosslinking can improve the

performance of hydrogel films. Ultimately, comparable efficacy will need to be demonstrated using natural, non-toxic crosslinkers.

Because the amino acids that bond covalently with glutaraldehyde are different from the ones involved in the ionic interactions with pectin, a chemical gel can be fabricated in gelatin simultaneously with the physical hydrogel described in the previous section. This combined approach to forming mixed hydrogels is unique and original.

Glutaraldehyde usually crosslinks proteins by reacting each aldehyde with two free unprotonated ϵ -amino groups, e.g. in lysine or hydroxylysine, to form Schiff's base bridges:



Schiff base crosslinking is acid-catalyzed but also requires unprotonated amine groups for the carbonyl-amine condensation, so pH is a pivotal factor affecting the course of gelatin crosslinking by aldehydes. At high pH, few amino groups are protonated, making more free amines available for crosslinking. Conversely, lowering the pH increases amine protonation until the reaction is completely precluded. The optimum pH of 4.2 observed for gelatin crosslinking should present a balance between these two requirements – enough acid for catalysis and enough unprotonated amines for reaction (Ege, 1999).

However, at this pH, unprotonated amines can only be provided by asparagine and glutamine, which gelatin does not contain. The three basic amino acids in gelatin (4% lysine, 1% hydroxylysine, and <1% histidine) are all charged at this pH. Thus, alternate reactions involving other functional groups must be active in the gelatin crosslinking.

It has been demonstrated that glutaraldehyde reacts with carboxylic, amide and other functionalities of proteins as well as amino groups (Friess, 1998). Crosslinking to alternate groups is strongly supported by the FT-IR spectra collected from gelatin films prepared with and without crosslinker at pH = 4.2, which showed no -C=N- absorption. We thus propose that the aldehyde groups of glutaraldehyde react with hydroxyl groups of hydroxyproline and hydroxylysine, which account for around 91 and 6.4 residues per 1000 residues in type A gelatin, respectively, by the path shown in Figure 4. In acidic media, the carbon alpha to the aldehyde becomes a highly reactive carbocation, which then undergoes nucleophilic attack by the -OH groups of hydroxyproline and hydroxylysine, and H^+ is regenerated in the medium. The final product is a hemiacetal stabilized by the low pH of the system and by the homogeneous charge distribution along the hemiacetal oxygen bridge. This reaction generates both short-range intramolecular bridges and long-range intermolecular crosslinks between hydroxyl groups of hydroxyproline extending out from the triple-helix (Tanzer, 1973). The potential for such long-range crosslinking is supported by previous studies demonstrating polymerization of glutaraldehyde in aqueous solution through aldol condensation comparable to the reaction with hydroxyproline (Nimni, Cheung, Strates, Kodama & Sheikh, 1988). The glutaraldehyde multimers so generated are able to cover larger distances between gelatin chains.

Tailoring cross-linking of biomolecules to generate a chemical hydrogel by one mechanism while a physical hydrogel is being formed in the same system by a second mechanism yields a new material that can properly be defined as a ‘permanent polyion-complex hydrogel’ – a three-dimensional

gel composed of a primary entangled network of chemically-crosslinked gelatin chains that connect and encase physical hydrogel regions in which pectin is linked to gelatin by ionic interactions (Figure 5). This multifaceted structure allows producing a hydrogel able to generate films with greatly improved mechanical properties (i.e. more elastic and able to sustain high load for a longer time) and pronounced water resistance. For example, our preliminary results (Farris, Schaich Liu, Cooke, Yam. Gelatin-pectin composite films from polyion complex hydrogels. *Journal of Agriculture and Food Chemistry*, submitted) demonstrated that elongation at break and tensile strength of gelatin-pectin films obtained from ‘permanent polyion-complex hydrogels’ were respectively 28% and 45% higher than in films obtained from an individual gelatin physical hydrogel. At the same time, water resistance in terms of swelling index calculated after 25 hours immersion was improved by approximately 90%.

Interpenetrating Polymer Network (IPN)

The matrix described above is characterized by physical entanglement among gelatin chains, which are also crosslinked via glutaraldehyde bridges. This structure increases mechanical strength, but the minor constituent (pectin) in the network interacts with gelatin only through electrostatic interactions that still can be easily disrupted in water. This presents a major problem for film stability. We propose that both long-term stability and mechanical strength of films formed from hydrogels can be increased by incorporating pectin as an individual three-dimensional network that can physically permeate the protein phase to form an interpenetrating network.

This approach more fully integrates the minor component into the matrix while at the same time limiting access from external media.

Increasing the concentration of pectin is the first step for generating an individual polysaccharide gel that will permeate and strengthen the overall network. To generate a full IPN, divalent cations (e.g. Ca^{++}) are also necessary to mediate crosslinking between excess pectin molecules and formation of an independent pectin hydrogel network dispersed within the gelatin-pectin hydrogel. When these two processes are combined with chemical crosslinking of gelatin, a unique new structure is produced, definable as a ‘permanent interpenetrating polyion-complex hydrogel’ (Figure 6).

Sequence of reaction is critical to control competitive reactions when forming complex interpenetrating networks. Pectin added too early is consumed in electrostatic interactions with gelatin. However, when a concentrated LM pectin solution ($\approx 5.0\%$) is added to a separately prepared gelatin-pectin complex physical hydrogel containing Ca^{++} ions, free pectin molecules migrate through the physical gel then become linked *in situ* by the Ca^{++} already present. This forms a full IPN of a pectin gel enmeshed with and dispersed throughout the gelatin-pectin physical hydrogel. Subsequent additions of glutaraldehyde will crosslink the gelatin molecules, entrapping the structured pectin hydrogel in the gelatin-pectin matrix (Figure 6).

It must be noted that LM pectin (DE = 7) is highly reactive with calcium ions at very low concentration (1 mg Ca^{2+} per g pectin), making control of the reaction extremely difficult to control (e.g. water quality is critical). Ca^{2+} also makes films stiff and brittle. These problems can be overcome by replacing conventional very low methoxyl pectins with amidated LM

pectins that have lower reactivity with calcium ions, making the IPN fabrication much easier to manipulate and control.

While the concept may seem rather simple, creating such IPNs is an important advance in gel network engineering that makes possible combining biopolymers with quite different behaviors to generate unique materials that have new composite properties while retaining some specific characteristics of individual component polymers (Kosmala et al., 2000). Applying gelatin and LM pectin in this approach combines clarity and flexibility from gelatin with toughness and mechanical strength from pectin. Using other biopolymers, relatively dense hydrogels can be combined in IPNs to generate stiff matrices with tougher mechanical properties, more widely controllable physical properties, and more efficient drug loading than are attainable with simple physical or chemical hydrogels (Hoare et al., 2008). The increasing interest towards this engineered approach is substantiated by the huge amount of research papers published in this last year on IPNs based on macromolecules other than gelatin and pectin: kappa-carrageenan-g-poly(methacrylic acid)/poly(N,N-diethylacrylamide) (Chen, Liu & Chen, 2009); poly(vinylpyrrolidone)-poly(urushiol) (Zheng, Weng, Huang, Hu, Qiao & Deng, 2009); collagen-phosphorylcholine (Liu et al., 2009); acrylic acid-starch (Li, Xu, Wang, Chen & Feng, 2009); poly(acrylamide)-carbohydrates (Vimala, Sivudu, Mohan, Sreedhar & Raju, 2009); silk fibroin-polyacrylamide (Mandal, Kapoor & Kundu, 2009); poly(acrylic acid-co-2-hydroxyethyl methacrylate)-poly(vinyl alcohol) (Yue, Sheng & Wang, 2009); dextran-gelatin (Liu & Chan-Park, 2009); N-carboxylethyl chitosan-photopolymerized poly (2-hydroxyethyl methacrylate) (Zhou et al., 2009); vinyl ester resin-modified lignin (Rosu,

Cascaval & Rosu, in press); poly(vinylacetate-co-hydroxyethyl methacrylate)-SiO₂ (Naghash, Massah & Arman, in press); chitosan-acrylamide-grafted hydroxyethylcellulose (Ahmed, Naik & Sherigara, in press).

Technical aspects of forming coatings and films from hydrogels

The theoretical approach illustrated in this review presents an innovative and alternative route to produce hydrogels that are biodegradable and economically viable starting materials for food packaging systems. Hydrogels produced using this approach also can be used in the same ways as existing single biopolymer films as direct-contact coatings (Krochta & De Mulder-Johnston, 1997; Petersen et al., 1999; Weber et al., 2002; Fernández, Cava, Ocio & Lagarón, 2008). The main difference is in how the hydrogels are handled. Coatings in direct contact with food are normally obtained by dipping or spraying followed by a drying step (Gennadios et al., 1997), while stand-alone films from biomacromolecules are produced by casting or extrusion.

Generating films by casting melted plastics or prepared biohydrogels involves spreading the film-forming solution onto flat, smooth and non-adhering substrates, followed by the removal of the solvent usually through a hot air flux. This method is most used in research because it is simple, practical, and does not require expensive equipment.

However, from a practical point of view, the affinity of biopolymers to water affects the removal of hydration water when hydrogels are used in films or coatings. In fact, this phase requires a dehydration step, but the time and the amount of energy required for the removal of water add little to the

overall process. Therefore, at industrial scales removal of the solvent (often 90 wt% of the total solution) is a major obstacle that makes casting technically and economically unrealistic. Here, extrusion provides faster throughput while requiring less energy to vaporize the water (Fishman, Coffin, Konstance & Onwulata, 2000). Extrusion also allows continuous operation with ready control of variables such as temperature, moisture, size/shape, etc. (Gómez-Guillén et al., 2008). However, because of the heat and shear stress involved, it may also adversely affect properties of films from biomacromolecules. For example, pig-skin gelatin films obtained by extrusion followed by heat-pressing had lower tensile strength and higher elongation and water vapor permeability (WVP) values than the same films obtained by solution-casting due to lower solvent evaporation rate for the cast films and larger and more numerous microvoids in the extruded films (Park, Scott Whiteside & Cho, 2008). These problems with degradation can be overcome by using milder extruder conditions, as has been demonstrated with pectin and gelatin as starting biomacromolecules (Fishman et al., 2000; Fishman et al., 2004; Fishman et al., 2006; Park et al., 2008).

A third way to exploit biomolecules for food packaging applications involves deposition of hydrogels onto plastic films as very thin coating layers in order to improve barrier properties, appearance, adhesion, wettability, scratch resistance, and frictional properties of the plastic substrate (Farris, Introzzi & Piergiovanni, 2009). In packaging, a film is defined as a layer having thickness not greater than 0.25 mm (American Society for Testing and Materials, 2008), while a coating is a very thin layer from hundreds of nanometers to a few microns thick. Coatings of this thickness can be readily produced in the laboratory manually or by

automatic applicators, followed by drying under hot air (Farris et al., 2009). Even though the biomolecules involved are strong water binders, drying time is short and does not add significantly to total processing time. Industrially, thin coatings of natural hydrogels are handled with the same procedures as synthetic coatings like varnishes, inks, polyvinyl alcohol, etc. Gravure coating affords a number of advantages over other coating deposition techniques (reverse roll coaters, calendar coaters, knife-over-roll coaters, blade coating, wirewound-rod coater) and seems to provide the best performance and cost-benefit overall, thus enhancing the marketability of innovative hydrogel solutions for food packaging applications (Farris, Piergiovanni, Ronchi & Rocca, 2008). The final choice of method depends strictly on the characteristics (especially rheological) of the hydrogel matrix that has to be coated.

Whatever the processing and film-forming method selected, there is one key difference between forming films from complex hydrogels as opposed to using conventional polymer solutions or generating non-film applications: to form a film, all the physical changes involved in forming the various forms of hydrogels must occur *in situ* during casting, extrusion, or depositing in order to ensure that the desired complex structure is in place in the final film. Extensive development of intermolecular interactions during hydrogel solution preparation would generate viscosities too high to distribute or matrices too solidified to flow. Thus, controlling the speed of the reactions in complex hydrogel formation during film manufacturing is undoubtedly the pivotal point for success. To accomplish this requires tight control over temperature, pH, moisture content, biopolymers ratios, and ionic strength of the system. Temperature, in particular, must be set

carefully during extrusion to allow a wide range of interactions and chemical reactions to take place as proteins denature and cross-link but at the same time avoid protein degradation during heat processing ([Hernandez-Hizquierdo & Krochta, 2008](#)). Analogously, during coating processes, the speed of the engraved roll in contact with the film-forming solution is critical. High speeds promote foam formation, especially when biomolecules like gelatin are used, resulting in opaque coatings with impaired barrier properties, more permeable to gas and vapors.

Conclusions

An overview of theoretical bases for formation of three types of hydrogels – physical hydrogels, chemical hydrogels, and interpenetrating polymer networks (IPN) – has been provided, and a process by which these three networks can be sequentially integrated to produce advanced packaging materials (e.g. films or coatings) has been outlined. The profitability of the resulting complex structure (‘permanent interpenetrating polyion-complex hydrogel’) is based on the fact that it combines benefits arising from the three component elements (physical hydrogel, chemical hydrogel, and interpenetrating polymer network) exploited simultaneously to overcome mechanical strength and water sorption limitations of simple biopolymer films.

Gelatin and pectin, used as model molecules to demonstrate the validity of the concepts presented, are suggested as excellent candidates for developing films for food packaging applications with desired clarity, mechanical strength, flexibility, low water sensitivity, and high thermal stability. This integrated approach can be extended to other biopolymers,

but since properties of hydrogels depend on the characteristics of starting biomolecules, modification of conditions and reaction sequences will be required to accommodate differences in intermolecular interaction mechanisms. The specific method described here will not produce comparable films when HM pectin is used in place of LM pectin or when ovalbumin is substituted for gelatin. Active associations must be identified and manipulated to develop new procedures tailored for the number, type, and location of charged groups, as well as crosslinking sites and regions of other interactions (e.g. hydrophobic) for each system.

Sequential integration of three forms of hydrogels marks significant progress in fabrication of new high-performance biopolymers and paves the way for generating a new family of hydrogels using biomaterials for food packaging. Options for tuning the influence or effects of any of the three hydrogel forms offer opportunities to tailor properties of composite matrices to a wide range of targeted food and non-food applications.

The composite hydrogel matrices described here also offer potential advantages in biodegradability, compostability, and bioassimilation of the original molecules as fertilizers and soil conditioners, and hence should reduce current environmental overloads from plastic packaging waste. Development and commercialization of these and other applications, however, will require some additional research. Effects of the protein crosslinking on film degradation must be assessed. Other issues in question include compatibility of these complex films with food, chemical and microbial inertness, sterilizability, and changes of molecular relationships within the system ‘food/packaging/environment’ during long-term storage. Use of these hydrogels for drug delivery, e.g. in active packaging or

pharmaceutical devices, will require a better understanding of the mechanisms governing the molecules release from these biomaterials to accurately predict the kinetic transport and release through the biopolymeric films. When these questions are answered, polyion-complex hydrogels should have a bright and profitable future.

Acknowledgments

Special thanks to Dr. Peter H. Cooke and Ms. Guoping Bao for technical assistance. We are also grateful to Dr. Songmiao Liang and Dr. Giovanna Catogno for their helpful suggestions.

References

AL-Kahtani Ahmed A., Bhojya Naik, H.S., & Sherigara B.S. Synthesis and characterization of chitosan-based pH-sensitive semi-interpenetrating

network microspheres for controlled release of diclofenac sodium. *Carbohydrate Research* (doi:10.1016/j.carres.2009.01.011).

Alvarez-Lorenzo, C., Concheiro, A., Dubovik, A.S., Grinberg, N.V., Burova, T.V., & Grinberg V.Y. (2005). Temperature-sensitive chitosan-poly(N-isopropylacrylamide) interpenetrated networks with enhanced loading capacity and controlled release properties. *Journal of Controlled Release*, *102*, 629-641.

Alves, V., Costa, N., Hilliou, L., Larotonda, F., Gonçalves, M., Sereno, A., & Coelho, I. (2008). Design of biodegradable composite films for food packaging. *Desalination*, *199*, 331-333.

ASTM. Standard Terminology Relating to Plastics. Designation D883-08. American Society for Testing and Materials.

Avena-Bustillos, R. J., Olsen, C. W., Olson, D. A., Chiou, B., Yee, E., Bechtel, P. J., & McHugh, T.H. (2006). Water vapor permeability of mammalian and fish gelatin films. *Journal of Food Science*, *71*, 202-207.

Axelos, M.A.V., & Thibault, J.F. (1991). The chemistry of low-methoxyl pectin gelation. In R.H. Walter, *The Chemistry and Technology of Pectin* (pp. 109-118). San Diego: Academic Press.

Baeva, M., & Panchev, I. (2005). Investigation of the retaining effect of a pectin-containing edible film upon the crumb ageing of dietetic sucrose-free sponge cake. *Food Chemistry*, *92*, 343-348.

Bédié, G.K., Turgeon, S.L., & Makhlof, J. (2008). Formation of native whey protein isolate-low methoxyl pectin complexes as a matrix for hydro-

soluble food ingredient entrapment in acidic foods. *Food Hydrocolloids*, 22, 836-844.

BeMiller, J.N. (1986). An introduction to pectins: structure and properties. In M.L. Fishman and J.J. Jen, *Chemistry and Function of Pectins* (pp. 2-11). Washington DC: American Chemical Society.

Bigi, A., Cojazzi, G., Panzavolta, S., Rubini, K., & Roveri, N. (2001). Mechanical and thermal properties of gelatin films at different degrees of glutaraldehyde crosslinking. *Biomaterials*, 22, 763-768.

Bordi, A., Paradossi, G., Rinaldi, C., & Ruzicka, B. (2002) Chemical and physical hydrogels: two case systems studied by quasi elastic light scattering. *Physica A*, 304, 119-128.

Braumer, K., Fischer, G., & Koepff, P. (1989). *European Patent 0 302 953 A1*.

Brodsky, B., & Ramshaw, J.A.M. (1997). The collagen triple-helix structure. *Matrix Biology*, 15, 545-554.

Cao, N., Fu, Y., & He, J. (2007). Preparation and physical properties of soy protein isolate and gelatin composite films. *Food Hydrocolloids*, 21, 1153-1162.

Chen, J., Liu, M., & Chen, S. (2009). Synthesis and characterization of thermo- and pH-sensitive kappa-carrageenan-g-poly(methacrylic acid)/poly(N,N-diethylacrylamide) semi-IPN hydrogel. *Materials Chemistry and Physics*, 115, 339-346.

- Chiellini, E. Cinelli, P., Grillo Fernandes, E., El-Refaie S. Kenawy, & Lazzeri, A. (2001). Gelatin-Based Blends and Composites. Morphological and Thermal Mechanical Characterization. *Biomacromolecules*, 2, 806–811.
- Chivukula, P., Dušek, K., Wang, D., Dušková-Smrčková, M., Kopečková, P., & Kopeček, J. (2006). Synthesis and characterization of novel aromatic azo bond-containing pH-sensitive and hydrolytically cleavable IPN hydrogels. *Biomaterials*, 27, 1140-1151.
- Coffin, D. R., & Fishman, M. L. (1993). Viscoelastic properties of pectin/starch blends. *Journal of Agricultural and Food Chemistry*, 41, 1192-1197.
- Coffin, D. R., & Fishman, M. L. (1994). Physical and mechanical properties of highly plasticized pectin/starch films. *Journal of Applied Polymer Science*, 54, 1311-1320.
- Dickinson, E. (2006). Colloid science of mixed ingredients. *Soft Matter*, 2, 642–52.
- Ege, S. (1999) Organic Chemistry: Structure and Reactivity – 4th Edition (p. 627). Boston: Houghton Mifflin.
- Elsabee, M.Z., Abdou, E.S., Nagy, K.S.A., & Eweis, M. (2008). Surface modification of polypropylene films by chitosan and chitosan/pectin multilayer. *Carbohydrate Polymers*, 71, 187-195.
- Erdohan, Z.O., & Turhan, K.N. (2005). Barrier and mechanical properties of methylcellulose-whey protein films. *Packaging Technology and Science*, 18, 295-302.

Farris, S., Introzzi, L., & Piergiovanni, L. (2009). Evaluation of a bio-coating as a solution to improve barrier, friction and optical properties of plastic films. *Packaging Technology and Science*, 22, 69-83.

Farris, S., Piergiovanni, L., Ronchi, G., & Rocca, R. International Patent WO 2008/075396 A1.

Fernández, A., Cava, D., Ocio, M.J., & Lagarón, J.M. (2008). Perspectives for biocatalysts in food packaging. *Trends in Food Science & Technology*, 19, 198-206.

Fishman, M.L., & Coffin, D.R. (1998). Mechanical, microstructural and solubility properties of pectin/poly(vinyl alcohol) blends. *Carbohydrate Polymers*, 35, 195-203.

Fishman, M. L., Coffin, D. R., Unruh, J. J., & Ly, T. (1996). Pectin/starch/glycerol films: blends or composites? *Journal of Macromolecular Science*, 33, 639-654.

Fishman, M.L., Coffin, D.R, Konstance, R.P, & Onwulata, C.I. (2000). Extrusion of pectin/starch blends plasticized with glycerol. *Carbohydrate Polymers*, 41, 317-325.

Fishman, M.L, Coffin, D.R., Onwulata, C.I., & Konstance, R.P. (2004). Extrusion of pectin and glycerol with various combinations of orange albedo and starch. *Carbohydrate Polymers*, 57, 401-413.

Fishman, M.L., Coffin, D.R., Onwulata, C.I, & Willett, J.L. (2006). Two stage extrusion of plasticized pectin/poly(vinyl alcohol) blends. *Carbohydrate Polymers*, 65, 421-429.

Friess, W. (1998). Collagen: biomaterial for drug delivery. *European Journal of Pharmaceutics and Biopharmaceutics*, 45, 113-136.

Gällstedt, M., Brottman, A., & Hedenqvist, M.S. (2005). Packaging-related properties of protein- and chitosan-coated paper. *Packaging Technology and Science*, 18, 161-170.

Gennadios, A., Hanna, M.A., & Kurth, L.B. (1997). Application of Edible Coatings on Meats, Poultry and Seafoods: A Review. *LWT - Food Science and Technology*, 30, 337-350.

Ghaffari, A., Navaee, K., Oskoui, M., Bayati, K., & Rafiee-Tehrani, M. (2007). Preparation and characterization of free mixed-film of pectin/chitosan/Eudragit® RS intended for sigmoidal drug delivery. *European Journal of Pharmaceutics and Biopharmaceutics*, 67, 175-186.

Gilsenan, P.M., Richardson, R.K., & Morris E.R. (2003). Associative and segregative interactions between gelatin and low-methoxy pectin: Part 1. Associative interactions in the absence of Ca²⁺. *Food Hydrocolloids*, 17, 723-737.

Gómez-Guillén, M.C., Pérez-Mateos, M., Gómez-Estaca, J., López-Caballero, E., Giménez B., & Montero, P. (2009). Fish gelatin: a renewable material for developing active biodegradable films. *Trends in Food Science & Technology*, 20, 3-16.

Gounga, M.E., Xu, S.-Y., & Wang, Z. (2007). Whey protein isolate-based edible films as affected by protein concentration, glycerol ratio and pullulan addition in film formation. *Journal of Food Engineering*, 83, 521-530.

Grant, G.T., Morris, E.R., Rees, D.A., Smith, P.J.C., & Thom, D. (1973). Biological interactions between polysaccharides and divalent cation: The egg-box model. *FEBS Letters*, 32, 195-198.

Guilbert, S., Gontard, N., & Gorris, L.G.M. (1996). Prolongation of the shelf-life of perishable food products using biodegradable films and coatings. *LWT-Food Science and Technology*, 29, 10-17.

Hernandez-Hizquierdo, V.M, & Krochta, J.M. (2008). Thermoplastic processing of proteins for film formation – A review. *Journal of Food Science*, 73, 30-39.

Hoare, T.R., & Kohane, D.S. (2008) Hydrogels in drug delivery: Progress and challenges. *Polymer*, 49, 1993-2007.

Hoffman, A.S. (2002). Hydrogels for biomedical applications. *Advanced Drug Delivery Reviews*, 43, 3-12.

Jaklenec, A., Wan, E., Murray, M. E., & Mathiowitz, E. (2008). Novel scaffolds fabricated from protein-loaded microspheres for tissue engineering. *Biomaterials*, 29, 185-192.

Jo, C., Kang, H., Lee, N. Y., Kwon, J. H., Byun, M. W. (2005). Pectin- and gelatin-based film: effect of gamma irradiation on the mechanical properties and biodegradation. *Radiation Physics and Chemistry*, 72, 745-750.

Jones, O.G., Decker, E.A., & McClements, D.J. (2009). Formation of biopolymer particles by thermal treatment of β -lactoglobulin–pectin complexes. *Food Hydrocolloids*, 23, 1312-1321.

- Kang, H.J., Jo, C., Kwon, J.H., Kim, J.H., Chung, H.J., & Byun, M.W. (2007). Effect of a pectin-based edible coating containing green tea powder on the quality of irradiated pork patty. *Food Control*, *18*, 430-435.
- Karim, A.A., & Bhat, R. (2009). Fish gelatin: properties, challenges, and prospects as an alternative to mammalian gelatins. *Food Hydrocolloids*, *23*, 563-576.
- Khademhosseini, A., & Langer, R. (2007). Microengineered hydrogels for tissue engineering. *Biomaterials*, *28*, 5087-5092.
- Khalil, A.H. (1999). Quality of french fried potatoes as influenced by coating with hydrocolloids. *Food Chemistry*, *66*, 201-208.
- Kosmala, J.D., Henthorn, D.B., & Brannon-Peppas, L. (2000). Preparation of interpenetrating networks of gelatin and dextran as degradable biomaterials. *Biomaterials*, *21*, 2019-2023.
- Kristo, E., & Biliaderis, C.G. (2006). Water sorption and thermo-mechanical properties of water/sorbitol-plasticized composite biopolymer films: caseinate–pullulan bilayers and blends. *Food Hydrocolloids*, *20*, 1057-1071.
- Krochta, J.M., & De Mulder-Johnston, C. (1997). Edible and biodegradable polymer films: challenges and opportunities. *Food Technology*, *51*, 61-74.
- LaCoste, A., Schaich, K.M., Zumbrunnen, D., Yam, K.L. (2005). Advancing controlled release packaging through smart blending. *Packaging Technology and Science*, *18*, 77-87.

- Lai, J.-Y., Lu, P.-L., Chen, K.-H., Tabata, Y., & Hsiue, G.-H. (2006). Effect of charge and molecular weight on the functionality of gelatin carriers for corneal endothelial cell therapy. *Biomacromolecules*, 7, 1836-1844.
- Langmaier, F., Mokrejs, P., Kolomaznik, K., & Mladek, M. (2008). Biodegradable packing materials from hydrolysates of collagen waste proteins. *Waste Management*, 28, 549-556.
- Lee, K.Y., Shim, J., & Lee, H.G. (2004). Mechanical properties of gellan and gelatin composite films. *Carbohydrate Polymers*, 56, 251-254.
- Leuenberger, B. H. (1991). Investigation of viscosity and gelation properties of different mammalian and fish gelatins. *Food Hydrocolloids*, 5, 353–361.
- Li, B., Kennedy, J.F., Jiang, Q.G., & Xie, B.J. (2006). Quick dissolvable, edible and heatsealable blend films based on konjac glucomannan – gelatin. *Food Research International*, 39, 544-549.
- Li, X., Xu, S., Wang, J., Chen, X., & Feng, S. (2009). Structure and characterization of amphoteric semi-IPN hydrogel based on cationic starch. *Carbohydrate Polymers*, 75, 688-693.
- Lin, C.-C., Metters, A.T. (2006). Hydrogels in controlled release formulations: network design and mathematical modeling. *Advanced Drug Delivery Reviews*, 58, 1379-1408.
- Liu, L.S., Fishman, M.L., Kost, J., & Hicks, K.B. (2003). Pectin-based systems for colon-specific drugdelivery via oral route. *Biomaterials*, 24, 3333-3343.

Liu, L.S., Won, Y.J., Cooke, P.H., Coffin, D.R., Fishman, M.L., Hicks, K.B., & Ma, P.X. (2004). Pectin/poly(lactide co-glycolide) composite matrices for biomedical applications. *Biomaterials*, *25*, 3201-3210.

Liu, L.S., Fishman, M.L., Hicks, K.B., & Kende, M. (2005). Interaction of various pectin formulations with porcine colonic tissues. *Biomaterials*, *26*, 5907-5916.

Liu, L.S., Liu, C.-K., Fishman, M.L., & Hicks, K.B. (2007). Composite Films from Pectin and Fish Skin Gelatin or Soybean Flour Protein. *Journal of Agricultural and Food Chemistry*, *55*, 2349-2355.

Liu, W.G., Li, X.W., Ye, G.X., Sun, S.J., Zhu, D., & Yao, K.D. (2004). A novel pH-sensitive gelatin–DNA semi-interpenetrating polymer network hydrogel. *Polymer International*, *53*, 675-680.

Liu, L., Kerry, J.F., & Kerry, J.P. (2007). Application and assessment of extruded edible casings manufactured from pectin and gelatin/sodium alginate blends for use with breakfast pork sausage. *Meat Science*, *75*, 196-202.

Liu, W., Deng, C., McLaughlin, C.R., Fagerholm, P., Lagali, N.S., Heyne, B., Scaiano, J.C., Watsky, M.A., Kato, Y., Munger, R., Shinozaki, N., Li, F., & Griffith, M. (2009). Collagen–phosphorylcholine interpenetrating network hydrogels as corneal substitutes. *Biomaterials*, *30*, 1551-1559.

Liu, Y., & Chan-Park, M.B. (2009). Hydrogel based on interpenetrating polymer networks of dextran and gelatin for vascular tissue engineering. *Biomaterials*, *30*, 196-207.

López-Caballero, M.E., Gómez-Guillén, M.C., Pérez-Mateos, & M. Montero, P. (2005). A chitosan–gelatin blend as a coating for fish patties *Food Hydrocolloids*, *19*, 303-311.

Maftoonazad, N., Ramaswamy, H.S., Moalemiyan, M., & Kushalappa, A.C. (2007). Effect of pectin-based edible emulsion coating on changes in quality of avocado exposed to *Lasiodiplodia theobromae* infection. *Carbohydrate Polymers*, *68*, 341-349.

Mandal, B.B., Kapoor, S., Kundu, S.C. Silk fibroin/polyacrylamide semi-interpenetrating network hydrogels for controlled drug release. *Biomaterials* (doi:10.1016/j.biomaterials.2009.01.040).

Mangiacapra, P., Gorrasi, G., Sorrentino, A., & Vittoria, V (2006). Biodegradable nanocomposites obtained by ball milling of pectin and montmorillonites. *Carbohydrate Polymers*, *64*, 516-523.

Mekhloufi, G., Sanchez, C., Renard, D., Guillemin, S., & Hardy, J. (2005). pH-induced structural transitions during complexation and coacervation of β -lactoglobulin and Acacia gum. *Langmuir*, *21*, 386-394.

Mesbahi, G., Jamalian, J., & Farahnaky, A. (2005). A comparative study on functional properties of beet and citrus pectins in food systems. *Food Hydrocolloids*, *19*, 2005, 731-738.

Miller, K.S., & Krochta, J.M. (1997). Oxygen and aroma barrier properties of edible films: a review. *Trends in Food Science and Technology*, *8*, 228-237.

Müller, C.M.O., Laurindo, J.B., & Yamashita, F. (2009). Effect of cellulose fibers addition on the mechanical properties and water vapor barrier of starch-based films. *Food Hydrocolloids*, 23, 1328-1333.

Nagahama, H., Maeda, H., Kashiki, T., Jayakumar, R., Furuike, T., & Tamura, H. (2009). Preparation and characterization of novel chitosan/gelatin membranes using chitosan hydrogel. *Carbohydrate Polymers*, 76, 255-260.

Naghash, H.J., Massah, A.R., & Arman, M. Silicone secondary cross-linked IPN based on poly(vinylacetate-co-hydroxyethyl methacrylate) and SiO₂ Progress in Organic Coatings (doi:10.1016/j.porgcoat.2008.12.005).

Neiryneck, N., Van der Meeren, P., Lukaszewicz-Lausecker, M., Cocquyt, J., Verbeken, D., & Dewettinck, K. (2007). Influence of pH and biopolymer ratio on whey protein–pectin interactions in aqueous solutions and in O/W emulsions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 298, 99-107

Nickerson, M.T., Farnworth, R., Wagar, E., Hodge, S.M., Rousseau, D., & Paulson, A.T. (2006). Some physical and microstructural properties of genipin-crosslinked gelatin–maltodextrin hydrogels. *International Journal of Biological Macromolecules*, 38, 40-44.

Nikolova, K., Panchev, I., & Sainova, S. (2005). Optical characteristics of biopolymer films from pectin and gelatin. *Journal of Optoelectronics and Advanced Materials*, 7, 1439-1444.

Nimni, M.E., Cheung, D., Strates, B., Kodama, M., & Sheikh, K. (1988). Bioprosthesis derived from crosslinked and chemically modified

collagenous tissues. In M.E. Nimni, *Collagen-Vol. III* (pp. 1-38). Boca Raton, FL: CRC Press.

Ofori-Kwakye, K., & Fell, J. T. (2003). Leaching of pectin from mixed films containing pectin, chitosan and HPMC intended for biphasic drug delivery. *International Journal of Pharmaceutics*, 250, 251-257.

Olsen, D., Yang, C., Bodo, M., Chang, R., Leigh, S., Baez, J., Carmichael, D., Perälä, M., Hämäläinen, E.R., Jarvinen, M., & Polarek, J. (2003). Recombinant collagen and gelatin for drug delivery. *Advanced Drug Delivery Reviews*, 55, 1547-1567.

Osés, J., Fabregat-Vázquez, M., Pedroza-Islas, R., Tomás, S.A., Cruz-Orea, A., & Maté, J.I. (2009). Development and characterization of composite edible films based on whey protein isolate and mesquite gum. *Journal of Food Engineering*, 92, 56-62.

Ou, Z., & Muthukumar, M. (2006). Entropy and enthalpy of polyelectrolyte complexation: Langevin dynamics simulations. *Journal of Chemical Physics*, 124, 154902.

Park, J.W., Whiteside, W.S., & Cho, S.Y. (2008). Mechanical and water vapor barrier properties of extruded and heat-pressed gelatin films. *LWT - Food Science and Technology*, 41, 692-700.

Petersen, K., Nielsen, P.V., Bertelsen, G., Lawther, M., Olsen, M.B., Nilsson, N.H., & Mortensen, G. (1999). Potential of biobased materials for food packaging. *Trends in Food Science and Technology*, 10, 52-68.

- Powell, D.A., Morris, E.L., Gidley, M.J., & Rees, D.A. (1982). Conformation and interactions of pectins. II. Influence of residues sequence on chain association in calcium pectate gels. *Journal of Molecular Biology*, *155*, 517-531.
- Qiu, Y., & Park, K. (2001). Environment-sensitive hydrogels for drug delivery. *Advanced Drug Delivery Reviews*, *53*, 321–339.
- Rosu, L., Cascaval, C.N., & Rosu, D. Effect of UV radiation on some polymeric networks based on vinyl ester resin and modified lignin. *Polymer Testing* (doi:10.1016/j.polymertesting.2009.01.004).
- Ryu, S.Y., Rhim, J.W., Roh, H.J., & Kim, S.S. (2002). Preparation and physical properties of zein-coated high amylase corn starch film. *LWT - Food Science and Technology*, *35*, 680-686.
- Schrieber, R., & Gareis, H. (2007). *Gelatine Handbook: Theory and Industrial Practice* (pp. 63-65). Weinheim: Wiley – VCH.
- Sikorski, Z.E., Scott, D.N., & Buisson, D.H. (1984). The role of collagen in the quality and processing of fish. *Critical Reviews in Food Science and Nutrition*, *20*, 301-343.
- Siracusa, V., Rocculi, P., Romani, S., & Dalla Rosa, M. (2008). Biodegradable polymers for food packaging: a review. *Trends in Food Science and Technology*, *19*, 634-643.
- Skotak, M., Leonov, A. P., Larsen, G., Noriega, S., & Subramanian, A. (2008). Biocompatible and biodegradable ultrafine fibrillar scaffold

materials for tissue engineering by facile grafting of L-lactide onto chitosan. *Biomacromolecules*, 9, 1902-1908.

Sorrentino, A., Gorrasi, G., & Vittoria, V. (2007). Potential perspectives of bio-nanocomposites for food packaging applications. *Trends in Food Science & Technology*, 18, 84-95.

Sriamornsak, P. (2003). Chemistry of Pectin and Its Pharmaceutica Uses: A Review. *Silpakorn University International Journal*, 3, 206-228.

Sriamornsak, P., & Nunthanid, J. (1998). Calcium pectinate gel beads for controlled release drug delivery: I. Preparation and in vitro release studies. *International Journal of Pharmaceutics*, 160, 207-212.

Sung, H.-W., Liang, I.-L., Chen, C.-N., Huang, R.-N., & Liang, H.-F. (2001). Stability of a biological tissue fixed with a naturally occurring crosslinking agent (*genipin*). *Journals of Biomedical Materials Research*, 55, 538-546.

Tanzer, M.L. (1973). Crosslinking of collagen. *Science*, 180, 561-566.

Te Nijenhuis, K. (1997). Thermoreversible networks: viscoelastic properties and structure of gels. *Advances Polymer Science*, 130, 1-267.

Terao, K., Nagasawa, N., Nishida, H., Furusawa, K., Mori, Y., Yoshii, F., & Dobashi, T. (2003). Reagent-free crosslinking of aqueous gelatin: manufacture and characteristics of gelatin gels irradiated with gamma-ray and electron beam. *Journal of Biomaterials Science-Polymer Edition*, 14, 1197-1208.

Tharanathan, R.N. (2003). Biodegradable films and composite coatings: past, present and future. *Trends in Food Science and Technology*, 14, 71-78.

Turgeon, S.L., Schmitt, C., & Sanchez, C. (2007). Protein-polysaccharide complexes and coacervates. *Current Opinion in Colloid & Interface Science*, 12, 166-178.

Villegas, R., O'connor, T. P., Kerry, J. P., & Buckley, D. J. (1999). Effect of gelatin dip on the oxidative and colour stability of cooked ham bacon pieces during frozen storage. *International Journal of Food Science and Technology*, 34, 385-389.

Vimala, K., Sivudu, K.S., Mohan, Y.M., Sreedhar, B., & Raju, K.M. (2009). Controlled silver nanoparticles synthesis in semi-hydrogel networks of poly(acrylamide) and carbohydrates: A rational methodology for antibacterial application. *Carbohydrate Polymers*, 75, 463-471.

Vishal Gupta, N.V., Satish, C.S., & Shivakumar, H.G. (2007). Preparation and characterization of gelatin-poly(methacrylic acid) interpenetrating polymeric network hydrogels as a pH-sensitive delivery system for glipizide. *Indian Journal of Pharmaceutical Sciences*, 69, 64-68.

Weber, C.J., Haugaard, V., Festersen, R., & Bertelsen, G. (2002). Production and applications of biobased packaging materials for the food industry. *Food Additives and Contaminants*, 19 (S), 172-177.

Wei, X., Sun, N., Wu, B., Yin, C., & Wu, W. (2006). Sigmoidal release of indomethacin from pectin matrix tablets: Effect of in situ crosslinking by calcium cations. *International Journal of Pharmaceutics*, 318, 132-138.

Wichterle, O., & Lim, D. (1960). Hydrophilic gels in biologic use. *Nature*, *185*, 117-118.

Yoo, S., Fishman, M.L., Savary, B.J., & Hotchkiss, A.T. (2003). Monovalent salt induced gelling system of enzymatically de-esterified pectin. *Journal of Agricultural and Food Chemistry*, *51*, 7410-7417.

Yue, Y., Sheng, X., & Wang, P. (2009). Fabrication and characterization of microstructured and pH sensitive interpenetrating networks hydrogel films and application in drug delivery field. *European Polymer Journal*, *45*, 309-315.

Zhang, Y.X., Wu, F.P., Li, M.Z., & Wang, E.J. (2005). pH switching on-off semi-IPN hydrogel based on crosslinked poly(acrylamide-co-acrylic acid) and linear polyallylamine. *Polymer*, *46*, 7695-7700.

Zheng, X.-L., Weng, J.B., Huang, Q.-M., Hu, B.-H., Qiao, T., & Deng, P. (2009). Fabrication of a stable poly(vinylpyrrolidone)/poly(urushiol) multilayer ultrathin film through layer-by-layer assembly and photo-induced polymerization. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *337*, 15-20.

Zhou, Y., Yang, D., Gao, X., Chen, X., Xu, Q., Lu, F., & Nie, J. (2009). Semi-interpenetrating polymer network hydrogels based on water-soluble N-carboxylethyl chitosan and photopolymerized poly (2-hydroxyethyl methacrylate). *Carbohydrate Polymers*, *75*, 293-298.

Zimet, P., & Livney, Y.D. (2009). Beta-lactoglobulin and its nanocomplexes with pectin as vehicles for ω -3 polyunsaturated fatty acids. *Food Hydrocolloids*, *23*, 1120-1126.

Figure Captions.

Figure 1. Overview of mechanisms for formation of physical and chemical hydrogels.

Figure 2. Sequential steps in association of peptide chains to form collagen.

Figure 3. Schematic illustration of the physical hydrogel formed by charge interactions between gelatin and pectin.

Figure 4. Proposed mechanism for reaction of glutaraldehyde with hydroxyl groups from hydroxyproline.

Figure 5. Schematic illustration of the gelatin-pectin ‘permanent polyion-complex hydrogel’.

Figure 6. Schematic illustration of the gelatin-pectin ‘full-IPN’.