

Sources of pectin, extraction and its applications in pharmaceutical industry – An overview

Pranati Srivastava^{1*} and Rishabha Malviya²

¹Advance Institute of Biotech and Paramedical Sciences, Near ALIMCO, 366 Naramau, G.T. Road, Kanpur, Uttar Pradesh, India

²Department of Pharmaceutical Technology, Meerut Institute of Engineering and Technology, Bypass Road- Baghpat Crossing, Meerut – 250005, Uttar Pradesh.

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Plant and their products have always been a source of various drugs and excipients used in pharmaceutical formulations. Pectin, a naturally occurring polysaccharide, has in recent years gained increased importance. The benefits of natural pectin are also appreciated by scientists and consumer due to its biodegradability. Pectin is the methylated ester of polygalacturonic acid and it is commercially extracted from citrus peels and apple pomace under mildly acidic conditions. Pectin is divided into two major groups on the basis of their degree of esterification. The association of pectin chains leads to the formation of the three dimensional networks giving gel formation. Since ages pectin is being used in the formulation of various dosage forms wherein it acts as a promising natural polymer for drug delivery. Pectin possesses several requisite characteristics to be used as polymer in drug development and release kinetics. In the present review we have compiled the major sources, extraction process and properties of pectin. The major fields and areas of its applications as a promising polymer have also been discussed. The pectin, by itself or by its gelling properties, is employed in pharmaceutical industry as a carrier for drug delivery to the gastrointestinal tract, such as matrix tablets, gel beads, film-coated dosage form. This review has also discussed the important chemistry and general properties of pectin, and its gel formation mechanism and properties.

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Introduction

Pectin (derived from Greek meaning - "congealed, and curdled") is a structural heteropolysaccharide contained in the primary cell walls of terrestrial plants. It was first isolated and described in 1825 by Heneri Bracannot. Pectin, a multifunctional constituent of cell wall is a high value functional food ingredient widely used as gelling agent and as stabilizer¹. It is produced commercially in form of white to light brown powder, mainly extracted from citrus fruits, and is used in food as a gelling agent particularly in jams and jellies. It is also used in fillings, sweets, as a stabilizer in fruit juices and milk drinks and as a source of dietary fiber². In plant cells, pectin consists of a complex set of polysaccharides that are present in most primary cell walls and particularly abundant in the non-woody parts of nearly all terrestrial plants. Pectin is present not only

in the primary cell walls but also in the middle lamella between plant cells where it helps to bind the cells together. The amount, structure and chemical composition of the pectin differs between plants, within a plant over time and in different parts of a single plant. During ripening, pectin is broken down by the enzymes pectinase and pectin esterase, resulting in the process where the fruit becomes softer. This is because the middle lamella which primarily consists of pectin breaks down and cells become separated from each other. A similar process of cell separation caused by pectin breakdown occurs in the abscission zone of the petioles of deciduous plants at the time of leaf fall².

Pectin is thus also a natural part of human diet, but does not contribute significantly to nutrition. As the literature reports, the daily intake of pectin from fruit and vegetables can be estimated to be around 5 g (where the consumption of approximately 500 g fruit and vegetable per day is estimated)³. In human digestion, pectin goes through the small intestine

*Correspondent author
E-mail: pranatiparul@gmail.com
Phone: +91 9452962662

more or less intact but is acted upon by microbial growth of large intestine. Pectin thus acts as a soluble dietary fibre¹. Consumption of pectin has been shown to reduce blood cholesterol levels. The mechanism appears to be an increase of viscosity in the intestinal tract, leading to a reduced absorption of cholesterol from bile or food³. In the large intestine and colon, microorganisms degrade pectin and liberate short-chain fatty acids that have favorable influence on health (also known as prebiotic effect).

Chemistry

In terms of structure, pectin is an essentially linear polysaccharide. Like most other plant polysaccharides, it is both polydisperse and polymolecular and its composition varies with the source and the conditions applied during isolation. In any sample of pectin, parameters such as the molecular weight or the contents of particular subunits differ even from molecule to molecule. The composition and structure of pectin are still not completely understood although pectin was discovered over 200 years ago¹. Through various studies it has been brought in notice that the structure of pectin is difficult to determine because pectin subunit composition can change during isolation from plants, storage, and processing of plant material⁵. At present, pectin is thought to consist mainly of D-galacturonic acid (GalA) units⁶, joined in chains by means of α -(1-4) glycosidic linkage. These uronic acids have carboxyl groups, which are naturally present as methyl esters and others which are commercially treated with ammonia to produce carboxamide groups (Figure 1)^{4,7}. Units range in number from a few hundred to about thousand saccharides in a chain-like configuration which corresponds to average molecular weights from about fifty thousand to one lack fifty thousand Dalton⁸. As the literature reports, into pectin backbone (made up of glycosides), galacturonic acid is replaced by (1-2)-linked L-rhamnose, at some distinguishing areas. From the rhamnose residues, side chains of various neutral sugars have been discovered to branch off. This type of pectin is termed as rhamnogalacturonan I. Here, up to every twenty fifth galacturonic acid in the main chain is replaced with rhamnose. The neutral sugars found in a pectin molecule are mainly D-galactose, L-arabinose and D-xylose, whose types and proportions vary with the origin of pectin⁹.

The X-ray fibre diffraction studies have reported that the galacturonan segments in the molecule of sodium pectate form helixes with three subunits per turn. The conformation of Galacturonic acid units as determined by NMR spectroscopy and referred from literatures is $4C_1$ ⁹. Calculations indicate that the helix is probably right-handed. It was indicated that X-ray fibre diffraction patterns of sodium and calcium pectates, pectic acids, and pectinic acids show the same helix structure, but the ways in which these helixes were arranged relative to each other in the crystals differ to various degrees. It has been suggested that helical pectinic acid molecules pack in a parallel arrangement, whereas the pectates pack as corrugated sheets of antiparallel helixes¹⁰.

Another structural type of pectin is rhamnogalacturonan II, which are comparatively less frequent complexes and a highly branched polysaccharide (Figure 2). This type of isolated pectin has reported molecular weight of 60-130,000 g/mol, varying with origin, extraction conditions and age of plant^{11, 12}. In nature, around 80% of carboxyl groups of galacturonic acid are esterified with methanol. This proportion is although reported to decrease more or less during pectin extraction. The ratio of esterified to non-esterified galacturonic acid determines the behavior of pectin in food applications. On this behalf, pectins are classified as high-ester or low-ester pectins; rather in short, HM (high-methoxy) versus LM (low-methoxy) pectins, with more or less than half of all the galacturonic acid esterified⁴.

Sources of pectin

Pectin is a complex mixture of polysaccharides that makes up about one third of the cell wall of dry substance of higher plants. Much smaller proportions of these substances are also found in the cell walls of grasses. The highest concentrations of pectin are found in the middle lamella of cell wall, with a gradual decrease as moving through the primary wall toward the plasma membrane¹³. Although pectin occurs commonly in most of the plant tissues, the number of sources that may be used for the commercial manufacture of pectin is limited. This is because; the ability of pectin to form gel depends on the molecular size and degree of esterification (DE). As discussed under the chemical structure section, the pectin from different sources does not have the same gelling ability due to variations in numerous numbers of parameters. Therefore, detection of a large quantity

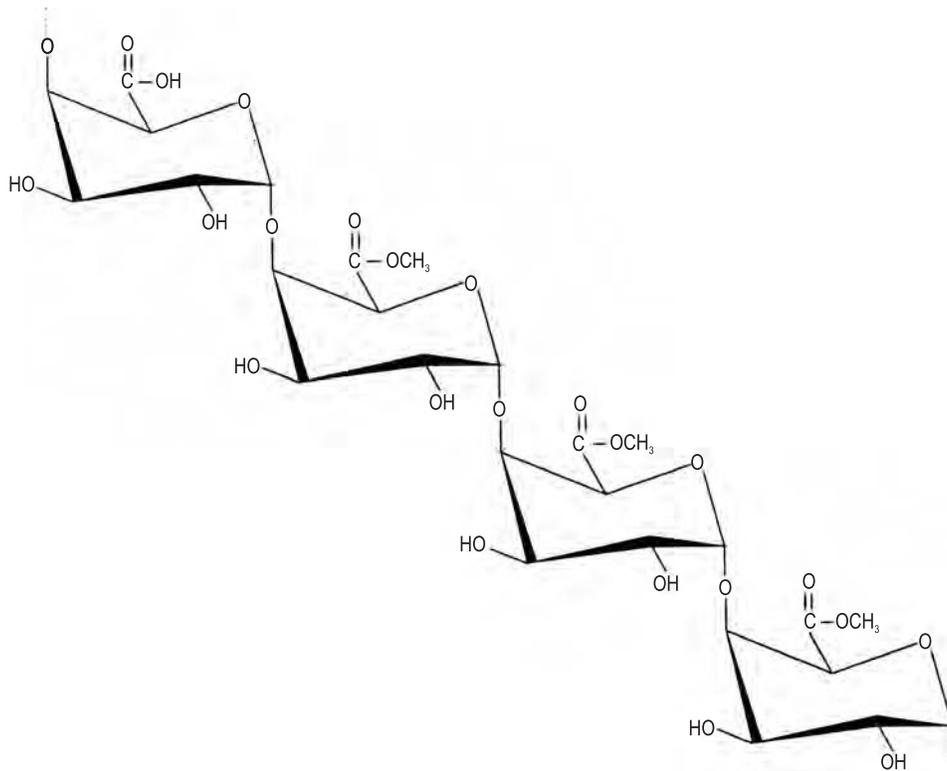


Fig. 1 – A repeating segment of pectin molecule and functional groups^{4,7}

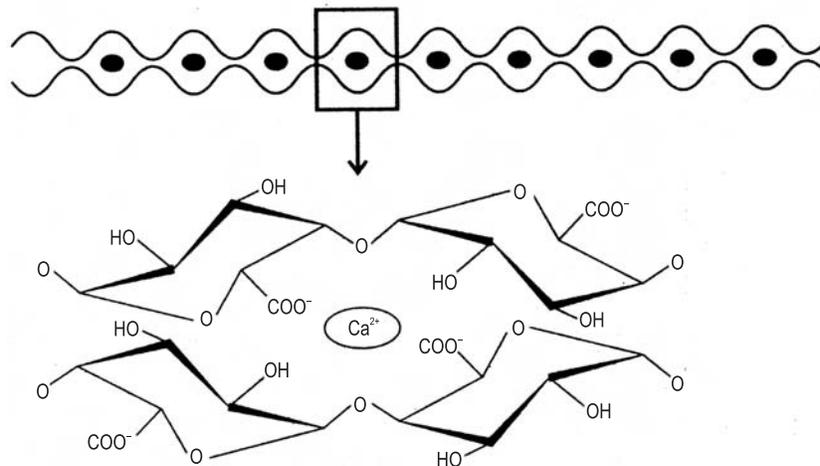


Fig. 2 – Schematic representation of calcium binding to polygalacturonate sequences³⁵

of pectin in a fruit alone is not in itself enough to qualify that fruit as a source of commercial pectin¹⁴. At present, commercial pectins are almost exclusively derived from citrus peel or apple pomace, both of which are by-products from juice manufacturing units. Apple pomace contains 10-15% of pectin on a dry matter basis. Citrus peel contains relatively higher i.e. 20-30% of pectin as compared to that of apple.

From an application point of view, citrus and apple pectins are largely equivalent². Among the physical properties, citrus pectins are light cream or light tan in colour whereas apple pectins are often darker. Alternative sources for pectin extraction include sugarbeet waste obtained from sugar manufacturing, sunflower heads (seeds used for edible oil), and mango waste^{15,16}.

Apples, quince, plums, gooseberries, oranges and other citrus fruits contain much more pectin as compared to soft fruits like cherries, grapes and strawberries contain little pectin¹. Typical levels of pectin in plants are (fresh weight): Apples, 1-1.5; Apricot, 1; Cherries, 0.4; Oranges, 0.5-3.5; Carrots, approx. 1.4 and Citrus peels, 30%.

Extraction procedures

The literature concerning the most commonly used method for extraction of pectin includes direct boiling and microwave heating¹⁷. Direct boiling is a conventional method of pectin extraction, which takes approximately two hours to obtain a good yield of pectin. Due to a relatively long period of direct heating, the extracted pectin undergoes thermal degradation^{7, 18}. Microwave heating extraction, on the other hand, takes no more than fifteen minutes to extract a satisfactory amount of pectin. Methods employing microwave heating are generally more effective in term of pectin yield and give better quality products as well. The yield of pectin also depends on the types of extraction solvents used and the use of extra chelating agents such as EDTA and CDTA which helps in releasing pectin from cell wall.

Water based extraction

The conventional water based extraction involves extracting the pectin using acidified water (pH up to 2)¹⁹ at temperature not more than 70°C. The assembly is run for 2-4 h duration and pectic substances are precipitated using ethanol or isopropyl alcohol.

A long list of various agents has been reported for the extraction of pectin from plant tissues. Extraction with the hot water is the simplest and oldest method for removing the pectic substances. The most commonly used acidifying materials are mineral acids including, sulfuric, hydrochloric and phosphoric acids. Many organic acids and their salts such as oxalic acid, ammonium oxalate, tartaric acid, polyphosphates, and many others have been also used. Meyer's and Rouse reported the use of different types of zeolites for the extraction of pectin. A very low yield of pectin obtained from dried orange peel was reported using Zeocarb as extractant at 85-90°C²⁰. Double extraction at 85-88°C for one hour using a cationic resin for the extraction of pectin from apple pomace has been reported to give higher yields and better gel strength of the product. IER has been extensively used in the purification and fractionalization of pectin.

Commercially, pectin is extracted by treating the raw material with hot dilute mineral acid at pH about two¹⁹. The precise length of extraction time varies with raw material, the type of pectin desired, and from one manufacturer to another. The hot pectin extract is separated from the solid residue as efficiently as possible. This is not easy since the solids are by now soft and the liquid phase are viscous. The viscosity increases with pectin concentration and molecular weight. There is a compromise between efficient extraction and solids separation and operating cost. The pectin extract may be further clarified by filtration through a filter aid. The clarified extract is then concentrated under vacuum. Powdered pectin can be produced by mixing the concentrated liquid from either apple or citrus with an alcohol (usually isopropanol). The pectin is separated as a stringy gelatinous mass, which is pressed and washed to remove the mother liquor, dried and grounded.

Microwave heating extraction

Microwave extraction has been carried out on a Milestone Ethos Microwave Lab Station. Additional solvents used in microwave extraction included 10% ethanol, 0.05M ethylenediamine tetra acetic acid, 1 M sodium hydroxide which was used to maintain pH up to 2 or less. Compared with water based extraction microwave heating extraction reduces the extraction period considerably. A fifteen minute microwave heating period is enough to extract almost the same amount of pectin as that obtained from water based extraction with a three hour extraction period^{16, 21}.

During microwave heating considerable pressure builds up inside a material. The high pressure then modifies the physical properties of material tissues, breaking down the cell structure and improving the capillary porous structure of tissues. This feature allows better penetration of extracting solvent into the tissues; improving the subsequent extraction of pectin. Microwave extraction also gave a higher rate and amount of extraction than the simple water based methods.

Extraction of pectin from raw papaya (*Carica papaya* Linn.) peel²² citrus peel^{23, 24} acerola, apple pomace²⁵, etc. has been reported using water based extraction procedure to procure pectic substances on large scale in industries²². Pectin extraction from pumpkin with the aid of microbial enzymes, at Saratov State Agrarian Vavilov University and Moscow State University of Applied Biotechnology

has also been a remarkable embankment for future and further scope of study in present field. Extraction and characterization of pectin from cocoa husks was done at Department di Scienza dei Material e Ingegneria Chimica and proved to be an emerging hope to search for such sources for pectin on industrial scale.

In the recent years supercritical fluid extraction technique, for extraction of natural polymers has come into existence. The relevancy of using the above technique for pectin extraction is yet to be proved over the other techniques available.

Molecular organization of extracted pectin in gels and solutions

In contrast to gels, images of pectin when casted onto mica discs reveal to form solutions. Isolated orange peel pectin at concentrations $>10 \mu\text{mg/ml}$ are reported to form integrated networks of strands which are separated by pores like those in sugar acid gels, but at lower concentrations, the strands get separated into linear subunits with a mean diameter (height) of 0.43 nm (SD=0.025) and mean length of 182 nm (SD=11). Commercially prepared pectin isolated from sugar beet pulp exhibited a similar concentration dependence of molecular association-dissociation, but in addition to strands, images revealed a conspicuous spherical component at nodes in the network of strands at high concentrations or similar nodes attached to the ends of isolated strands at low concentrations. The clear transition from networks to subunits in solutions and the similarity in structure between the concentrated solution and hydrated gels of diverse pectin suggest that the organization of molecules in the fluid state is a precursor of the organization in gels²⁶.

General properties of pectin

Pectin is soluble in pure water. Monovalent cation (alkali metal) salts of pectinic and pectic acids are usually soluble in water; di- and trivalent cations salts are weakly soluble or insoluble. Dry powdered pectin, when added to water, has a tendency to hydrate very rapidly, forming clumps. These clumps consist of semi dry packets of pectin contained in an envelope of highly hydrated outer coating⁴. Further solubilization of such clumps is very slow. Clump formation can be prevented by dry mixing pectin powder with water-soluble carrier material or by the use of pectin having improved dispensability through special treatment

during manufacturing²⁷. Dilute pectin solutions are Newtonian but at a moderate concentration, they exhibit the non-Newtonian, pseudo plastic behaviour characteristics. As with solubility, the viscosity of a pectin solution is related to the molecular weight, degree of esterification, concentration of the preparation, and the pH and presence of counter ions in the solution. Viscosity, solubility, and gelation are generally related. For example, factors that increases gel strength will increase the tendency to gel, decrease solubility, and increase viscosity, and vice versa¹⁸. These properties of pectins are a function of their structure. As such, monovalent cation salts of pectins are highly ionized in solution, and the distribution of ionic charges along the molecule tends to keep it in extended form by reason of coulombic repulsion²⁸. Furthermore, this same coulombic repulsion between the carboxylate anions prevents aggregation of the polymer chains. The number of negative charges is, determined by the DE. In addition, each polysaccharide chain, and especially each carboxylate group, will be highly hydrated²⁹. Solutions of monovalent salts of pectins exhibit stable viscosity because each polymer chain is hydrated, extended, and independent. As the pH is lowered, ionization of the carboxylate groups is suppressed, and this results in a reduction in hydration of the carboxylic acid groups. As a result of reduced ionization, the polysaccharide molecules no longer repel each other over their entire length and can associate and form a gel. Apparent pK-values (pH at 50% dissociation) vary with the DE of the pectin²⁹; a 65% DE pectin has an apparent pK of 3.55, while a 0% DE pectic acid has an apparent pK of 4.10. However, pectins with increasingly greater degrees of methylation will gel at somewhat higher pH, because they have fewer carboxylate anions at any given pH. Dissolved pectins are decomposed spontaneously by de-esterification as well as by depolymerisation; the rate of this decomposition depends on pH, water activity, and temperature. In general, maximum stability is found at pH 4. The presence of sugar in the pectin solution has a certain protective effect while elevated temperatures increase the rate of degradation⁴. At low pH-values and elevated temperatures degradation due to hydrolysis of glycosidic linkages is observed. De-esterification is also favored by low pH. By de-esterification a HM-pectin becomes slower setting or gradually adapts LM-pectin characteristics. At near-

to-neutral pH (5-6), HM-pectin is stable at room temperature only. As the temperature (or pH) increases, so-called elimination starts which results in chain cleavage and very rapid loss of viscosity and gelling properties. LM-pectins show a somewhat better stability at these conditions. At alkaline pH-values pectin is rapidly de-esterified and degraded even at room temperature^{30, 31}.

Powdered HM-pectins slowly lose their ability to form gels if stored under humid or warm conditions while LM-pectins are more stable and loss should not be significant after one year storage at room temperature³⁰.

Other natural polymers having pharmaceutical application

Since ages there has been use of various other natural polymers in formulation of dosage form and contribute a significant role in drug delivery. In recent years, plant derived polymers have evoked tremendous interest due to their diverse pharmaceutical applications such as diluents, binder, disintegrant in tablets, thickeners in oral liquids, protective colloids in suspensions, gelling agents in gels and bases in suppository³¹. Natural gums and mucilage are well known for their medicinal use. Mucilage and gums of plants like *Aloe barbadensis* Mill., *Hibiscus rosa-sinensis* Linn. and *Trigonella foenum-graceum* Linn. have proved to be efficient polymers in designing of various pharmaceutical dosage forms. Fenugreek mucilage and other polymers have proved to be efficient in drug delivery³²⁻³⁶. Polymers like tamarind gum, guar gum, gum Arabic and chitosan have been reported in use since long³⁷.

Applications of pectin

Mucoadhesive polymer

Different types of pectin were characterized for gastrointestinal (GI) mucoadhesion⁶. The mucoadhesive process involved in the formation of bioadhesive bonds has been described in three steps: (i) wetting and swelling of polymer to permit intimate contact with biological tissue, (ii) interpenetration of bioadhesive polymer chain and entanglement of polymer and mucin chains and (iii) formation of weak chemical bonds between entangles chains^{38, 39}. Thus this property of pectin is exploited in preparation of mucoadhesive patches in combination with other mucoadhesive polymer like carbopol and chitosan.

Gelling agent, thickener and water binder

Pectins are mainly used as gelling agents, but can also act as thickener, water binder and stabilizer. Low methoxyl pectins (<50% esterified) form thermoreversible gels in the presence of calcium ions and at low pH (3-4.5) whereas high methoxyl pectins rapidly form thermally irreversible gels in the presence of sufficient (for example, 65% by weight) sugars such as sucrose and at low pH (<3.5); the lower the methoxyl content, the slower the set⁴⁰. The degree of esterification can be incompletely reduced using commercial pectin methylesterase, leading to a higher viscosity and firmer gelling in the presence of Ca²⁺ ions. Highly (2-O- and/or 3-O-galacturonic acid backbone) acetylated pectin from sugar beet is reported to gel poorly but have considerable emulsification ability due to its more hydrophobic nature, but this may be due to associated protein impurities. But sugar beet pectin has different chemical features than citrus pectin, so it could find new uses, especially in industrial products¹⁸.

As with other viscous polyanions such as carrageenan, pectin may be protective towards milk casein colloids, enhancing the properties (foam stability, solubility, gelation and emulsification) of whey proteins whilst utilizing them as a source of calcium. But the main use for pectin still remains as a gelling agent, thickening agent and stabilizer in food¹. The classical application is giving the jelly-like consistency to jams or marmalades, which would otherwise be sweet juices. For household use, pectin is an ingredient in jelling sugar (sometimes sold as "sugar with pectin") where it is diluted to the right concentration with sugar and some citric acid to adjust pH.

In some countries, pectin is also available as a solution or an extract, or as a blended powder, for home jam making⁴¹. For conventional jams and marmalades that contain above 60% sugar and soluble fruit solids, high-ester pectins are used. With low-ester pectins and amidated pectins less sugar is needed, so that diet products can be made. Pectin can also be used to stabilize acidic protein drinks, such as drinking yogurt, and as a fat substitute in baked goods. Typical levels of pectin used as a food additive is between 0.5-1.0% this is about the same amount of pectin as in fresh fruit.

Pectin in medicine and pharmaceutical industry

In medicine, pectin increase viscosity and volume of stool that it is used against constipation and diarrhoea. Until 2002, it was one of the main ingredients used in Kaopectate, along with kaolinite. Pectin is also used in throat lozenges as a demulcent. In cosmetic products, pectin acts as stabilizer. Pectin is also used in wound healing preparations and especially in medical adhesives, such as colostomy devices.

As dietary fibre: In ruminant nutrition, depending on the extent of lignification of the cell wall, pectin is up to 90% digestible by bacterial enzymes. Ruminant nutritionists recommend that the digestibility and energy concentration in forages can be improved by increasing pectin concentration in the forage⁴².

As natural prophylactic: Pectin acts as a natural prophylactic substance against poisoning with toxic cations. It has been shown to be effective in removing lead and mercury from the gastrointestinal tract and respiratory organs. When injected intravenously, pectin shortens the coagulation time of drawn blood, thus being useful in controlling hemorrhage or local bleeding⁴⁵. Pectin and combinations of pectin with other colloids have been used extensively to treat diarrhoea, especially in infants and children. Although a bactericidal action of pectin has been proposed to explain the effectiveness of pectin treating diarrhoea, most experimental results do not support this theory. However, some evidence suggests that under certain *in-vitro* conditions, pectin may have a light antimicrobial action toward *Echerichia coli*¹⁴.

In treatment of overeating: Pectin reduces rate of digestion by immobilizing food components in the intestine. This results in less absorption of food. The thickness of the pectin layer influences the absorption by prohibiting contact between the intestinal enzyme and the food, thus reducing the latter's availability⁴⁴⁻⁴⁶. Due to its large water binding capacity pectin gives a feeling of satiety, thus reducing food consumption⁴⁷. Experiments showed a prolongation of the gastric emptying half-time from 23 to 50 minutes of a meal fortified with pectin. These attributes of pectin are used in the treatment of disorders related to overeating^{48, 49}.

In controlled release formulations: Pectin hydrogels have been used in tablet formulations as a binding agent and have been used in controlled-release matrix tablet formulations. Recently, it has been investigated HM-pectins for their potential value in controlled

release matrix formulations⁵⁰⁻⁵³. The application of a binary polymer system, i.e. HM-pectin and hydroxypropyl methylcellulose, in drug release rate modulation for oral administration was studied⁵⁴. Pectin beads prepared by the ionotropic gelation method⁵⁵ were used as a sustained release drug delivery system. However, the use of these beads has some drawbacks due to their rapid *in vitro* release. By changing the DE of LM-pectin⁵⁶, the drug release pattern from calcium pectinate gel beads can be modified. Since pectin can react with calcium ions, calcium pectinate has been investigated as an insoluble hydrophilic coating for sustained release delivery by interfacial complexation process. The spherical pellets, which contain calcium acetate, were prepared using an extrusion spheronisation method and then coated in a pectin solution. An insoluble and uniform coating of calcium pectinate gel was formed around the pellets. The use of pectin to develop other oral controlled release drug delivery systems has been reported by some authors. Srivastava et al demonstrated the use of orange peel derived pectin as an efficient polymer to act as good binding agent during the formulation of dosage form. The contrary fact reveals the truth that at different concentrations mango peel derived pectin acted as superdisintegrating agent in dosage form formulations as shown by recent studies⁵⁷.

In site specific targeting: Pectin has a promising pharmaceutical uses and is presently considered as a carrier material in colon-specific drug delivery systems⁵⁸ (for systemic action or a topical treatment of diseases such as ulcerative colitis, Crohn's disease, colon carcinomas), as indicated by the large number of studies published over the last few years^{26, 31}. The potential of pectin or its salt as a carrier for colonic drug delivery was also studied. The rationale for this is that pectin and calcium pectinate will be degraded by colonic pectinolytic enzymes⁵⁹, but will retard drug release in the upper gastrointestinal tract due to its insolubility and because it is not degraded by gastric or intestinal enzymes⁶⁰. It has also been demonstrated that pectin-degrading bacteria, *Klebsiella oxytoca*, could adhere to a film casted of low methoxylated pectin. The ability of the bacteria to adhere to the films, however, was not correlated with their ability to degrade pectin. When the dissolution of pectin matrix tablets was analyzed with and without *K. oxytoca*, a significant retardation in the dissolution rate was observed in the presence of *K. oxytoca*, suggesting the

formation of a biofilm on the matrix or sedimentation of insoluble pectin salts.

Thus, pectin is an interesting constituent for pharmaceutical use, e.g. as a carrier of a variety of drugs for controlled release applications. Many techniques have been used to manufacture the pectin-based delivery systems, especially ionotropic gelation and gel coating. These simple techniques, together with the very safe toxicity profile, make pectin an exciting and promising excipient for the pharmaceutical industry for present and future applications⁶¹.

Although the fact remains in the concept that pectin is one of the most versatile stabilizers available. Product and application development by the major pectin producers has over the years resulted in a large expansion of the opportunities and applicability of pectin.

Conclusion

Over the years the positive public connotation of pectin has proven helpful in its widespread use and this may be a contributing factor to the growing interest in investigating pectin for possible direct health benefits and thus applications in regulated non-food segment as well as in functional foods and nutraceuticals. Dealing with various sources, it has been found that pectin can easily be obtained from various natural sources but good and high grade of yield depends upon some major sources. Conventionally, various extraction procedures used have reported different percentage yield, but still the most reliable remains Soxhlet water based extraction. Pectin finds enormous number of applications such as mucoadhesive polymer, gelling agent and also in medical and pharmaceutical field. This wide range of applications explains the need for many different types of commercial pectins, which are sold according to their application, grade and quality.

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