

Chapter 1

Classification of Natural Polymers

Natural polymers by themselves are a class of polymers from nature (**plants or animals**).

They include mainly **carbohydrates** and **proteins** which exist in plants and animals providing mainly structural support

Polymers existing in nature can be grouped into six main classifications with respect to their sources:

Proteins, polysaccharides polynucleotides, polyisoprenes, polyesters, and lignin

Table 1.1 List of some polysaccharides from various sources

Source	Polymer
Cells walls of plants	Pectin
Seeds and roots	Galactomannans
Seaweeds	Carragenans, alginates, agar
Animal cell walls	Hyaluronan
Shells of aquatic animals	Chitin
Wood	Cellulose, lignin, hemicellulose
Skins and bones of animals and scales of fish	I Gelatin
Bacteria	Xanthan, hyaluronan, gellan
Fungi	Cardlan, scleroglucan, schizophyllan

- **Polysaccharides**
- These are homopolymers of glucose or amino sugars linked by glycoside bonds.
- Polysaccharides are of various types depending on their structure or function. **In terms of function** there are three main types;
- ***storage polysaccharides*** such as starch and glycogen, ***structural polysaccharides*** such as cellulose and chitin, and
- ***gel forming polysaccharides*** such as alginic acid and mucopolysaccharides.
- They can also be branched or straight chained polymers, ionic or nonionic (cationic and anionic) polymers.

- The major **storage polysaccharides** are
- starch (amylase and amylopectin) and glycogen, while the most common structural ones are **chitin and cellulose.**

Chitin being the structural polysaccharides in some animals such as crustaceans while cellulose is the main structural component of plants alongside hemicelluloses, pectin, and lignin.

Hyaluronan is another structural polysaccharide found in human cells. Hyaluronic acid is also an anionic polysaccharide.

- **Cellulose**

- **Cellulose as the principal component of plant cell wall makes up about half of the biomass of photosynthetic organisms, thus making cellulose possibly the most abundant molecule on earth.**
- **Alongside other components such as lignin, pectin, and hemicelluloses, it makes up the cell wall which is the distinguishing difference between animal and plant cells**



Middle
Lamella

Primary
Cell Wall

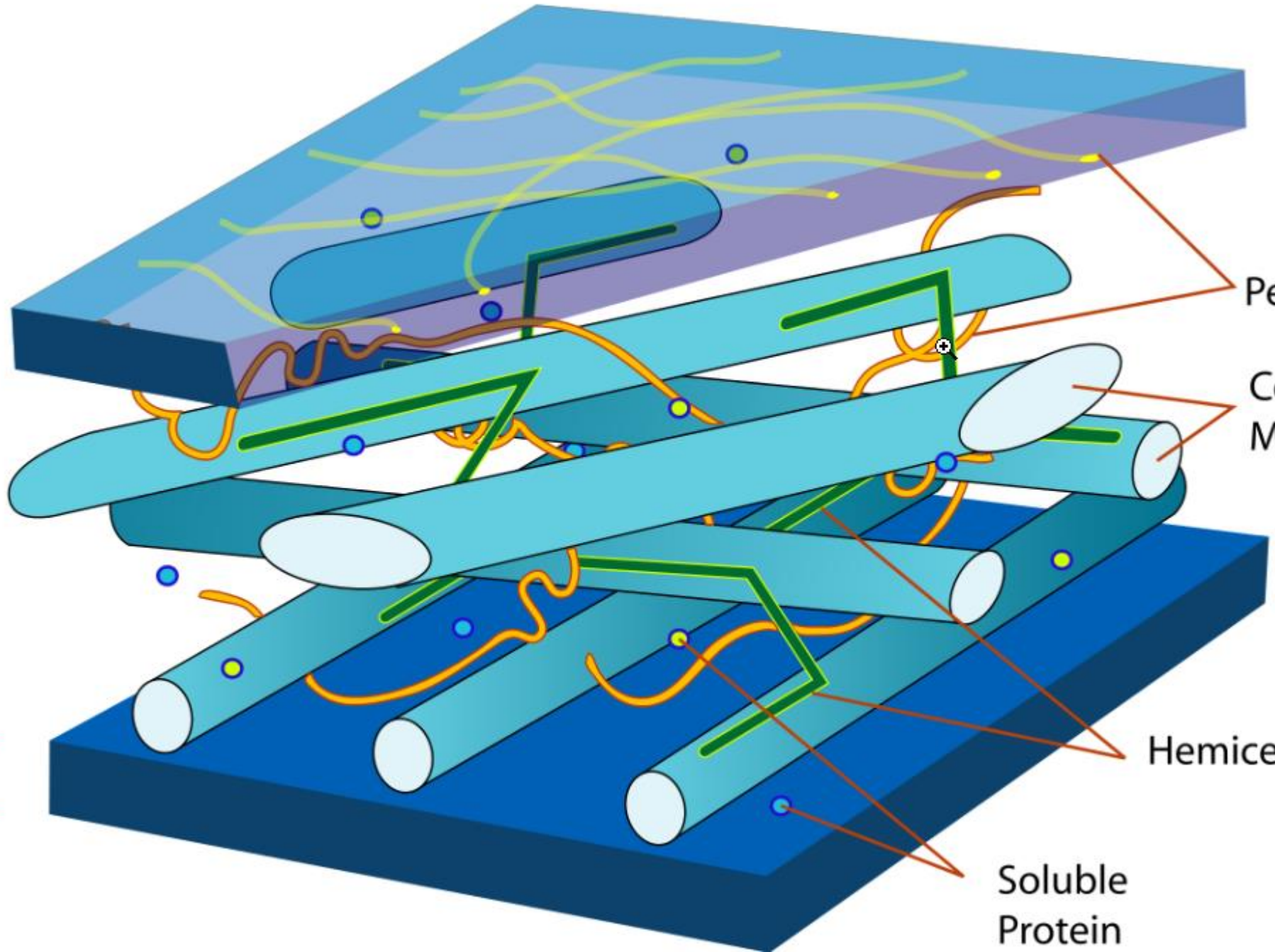
Plasma
Membrane

Pectin

Cellulose
Microfibril

Hemicellulose

Soluble
Protein

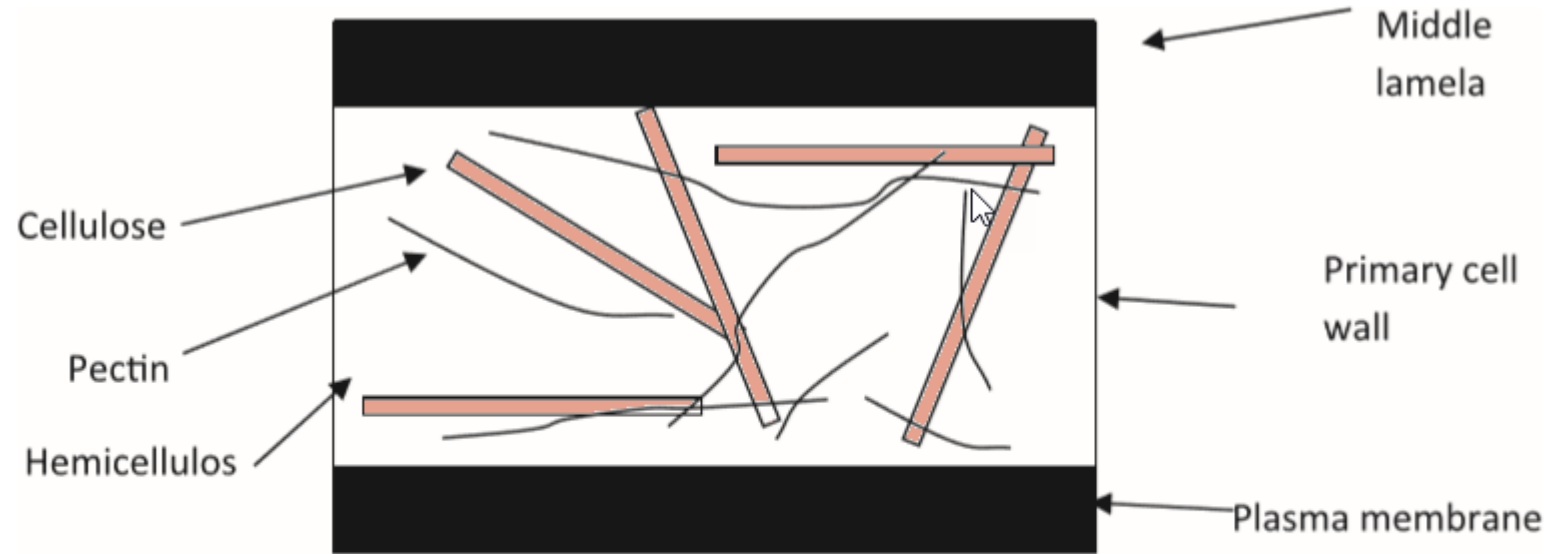


- **cellulose** has been abundantly sourced from wood, cotton, hemp, linen, jute, kenaf, sugar beet cereal straws, flax and is widely exploited for various industrial applications.

Applications of cellulose range from clothing pulp and paper to food conferring huge economic relevance.

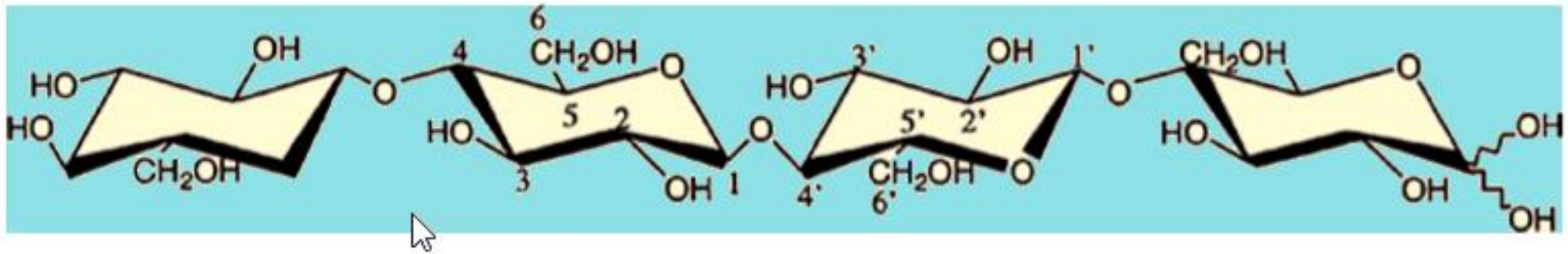
Other sources of cellulose include bacteria (e.g., *Acetobacter*), algae (e.g., *Valonia* and *Microdictyon*), and marine animals of the *Ascite* family.

Cellulose cannot be digested by the human body, however, animals, in particular ruminants, can digest cellulose. It is also water insoluble.



Schematic representation of plant cell wall showing the various polysaccharide components.

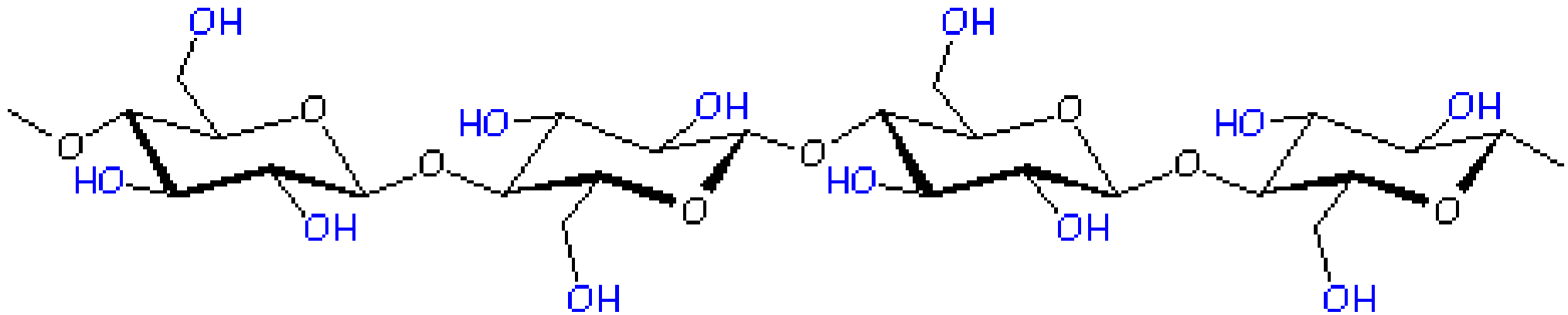
The fundamental formula of the cellulose structure was established by Willstatter and Zechmeister (1913) while further work (Irvine and Hirst 1923) resulted in the presently accepted concept of cellulose as macromolecular. By late 1931, following the numerous various work done on cellulose the primary structure had already been established as a linear homopolymer of glucose residues with D configuration linked by β -(1 \rightarrow 4) glycosidic linkage



Schematic representation of cellulose chain.

Natural Polymers: Polymers in Plants

Cellulose



Cellulose, a linear polymer of D-glucose units (two are shown)
linked by
 $\beta(1 \rightarrow 4)$ -glycosidic bonds.

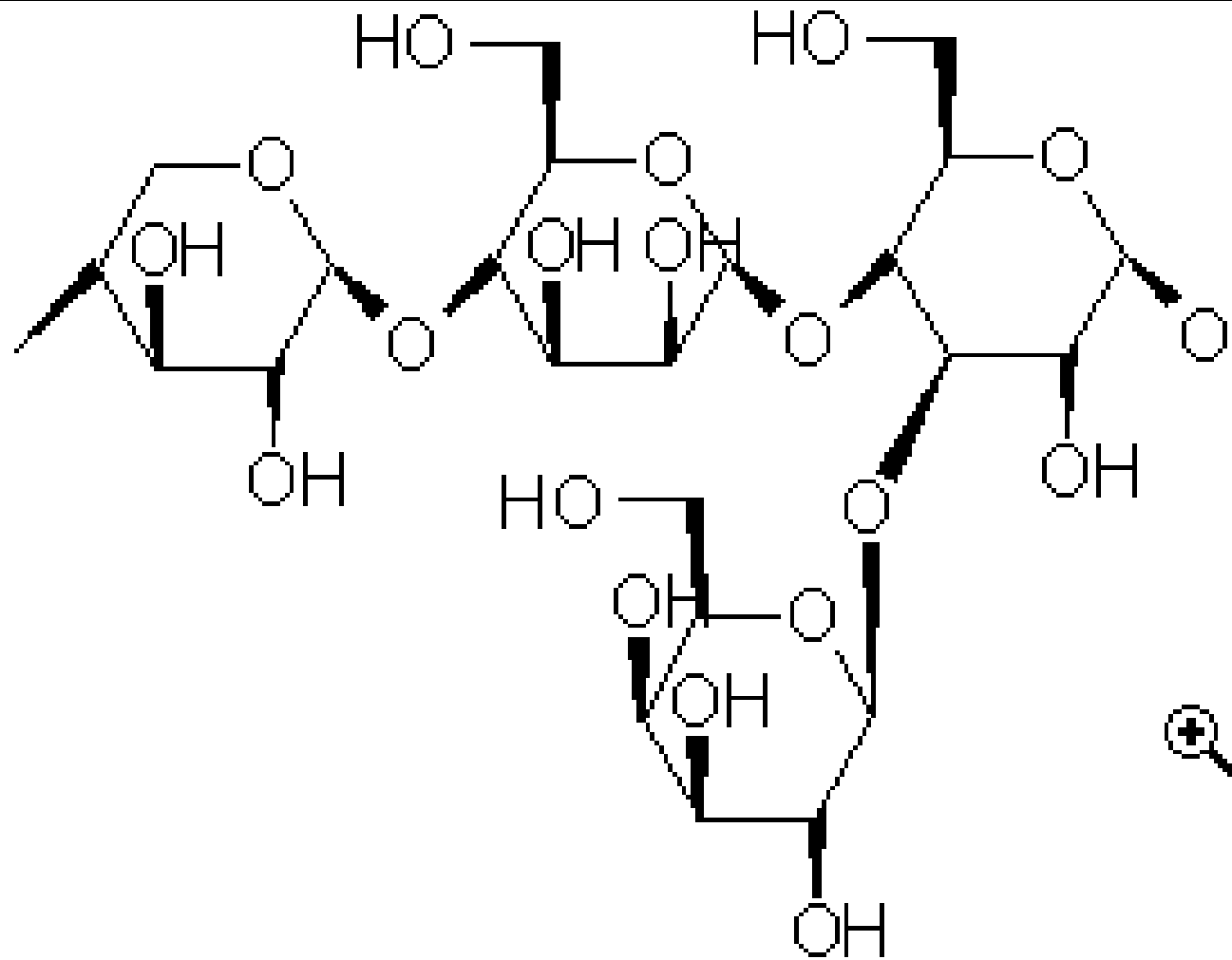
• Hemicelluloses

Another major component of plant cells is hemicelluloses which form a matrix for the cellulose microfibrils.

Hemicelluloses are made up of a variety of molecules such as xyloglucans, xylans, mannans, and $\beta(1-3)$ - $\beta(1-4)$ -glucans forming a matrix around the cellulose microfibrils. They are usually molecularly bonded to the cellulose microfibrils via forces such as hydrogen bonds, van der Waals forces, and other molecular interactions.

Hemicelluloses also serve other functions such as cell signaling or acting as reserves for metabolism.

Unlike cellulose, **hemicelluloses** consist of shorter chains – 500–3,000 sugar units as opposed to 7,000–15,000 glucose molecules per polymer in cellulose. In addition, **hemicellulose** may be branched polymers, while cellulose is unbranched.



- Xylose - $\beta(1,4)$ - Mannose - $\beta(1,4)$ - Glucose -
- $\alpha(1,3)$ - Galactose

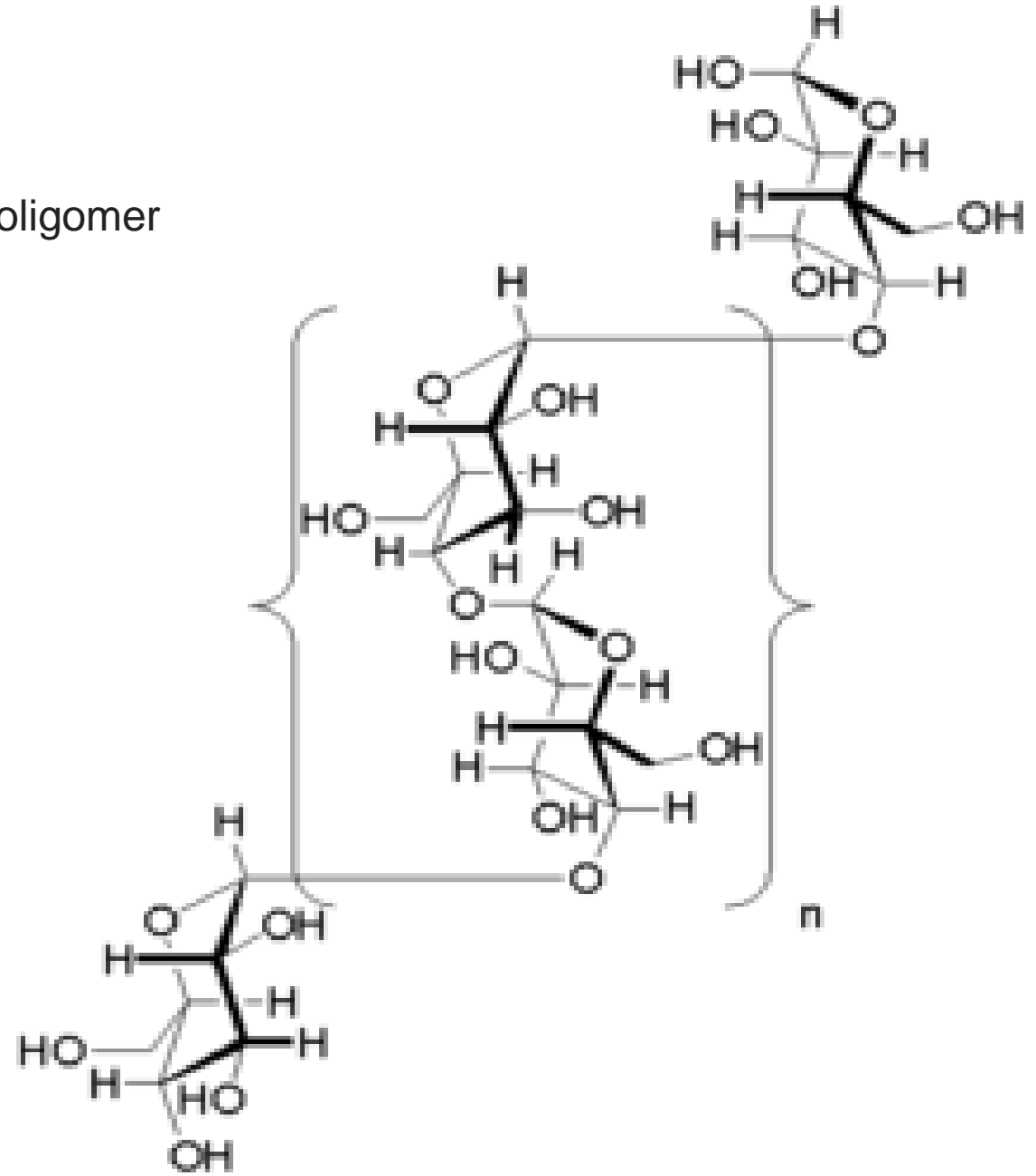
Hemicellulose

- While **cellulose** is strong and relatively chemically stable straight chain **crystalline polysaccharide**,
- **hemicelluloses** have an **amorphous** branched structure and little mechanical strength. They also have a more random nature with shorter chain.

Glycogen

is another type of **storage polysaccharide**. It is highly branched and compact like other storage polysaccharides such as amylopectin except with **more branching and compactness**. Glycogen exists in the cytoplasm of animal cells where it serves as **the main storage form for glucose**. Albeit very significant for body metabolism, glycogen has no industrial application and is only mentioned here for completeness.

1,4- α -glycosidic linkages in the glycogen oligomer



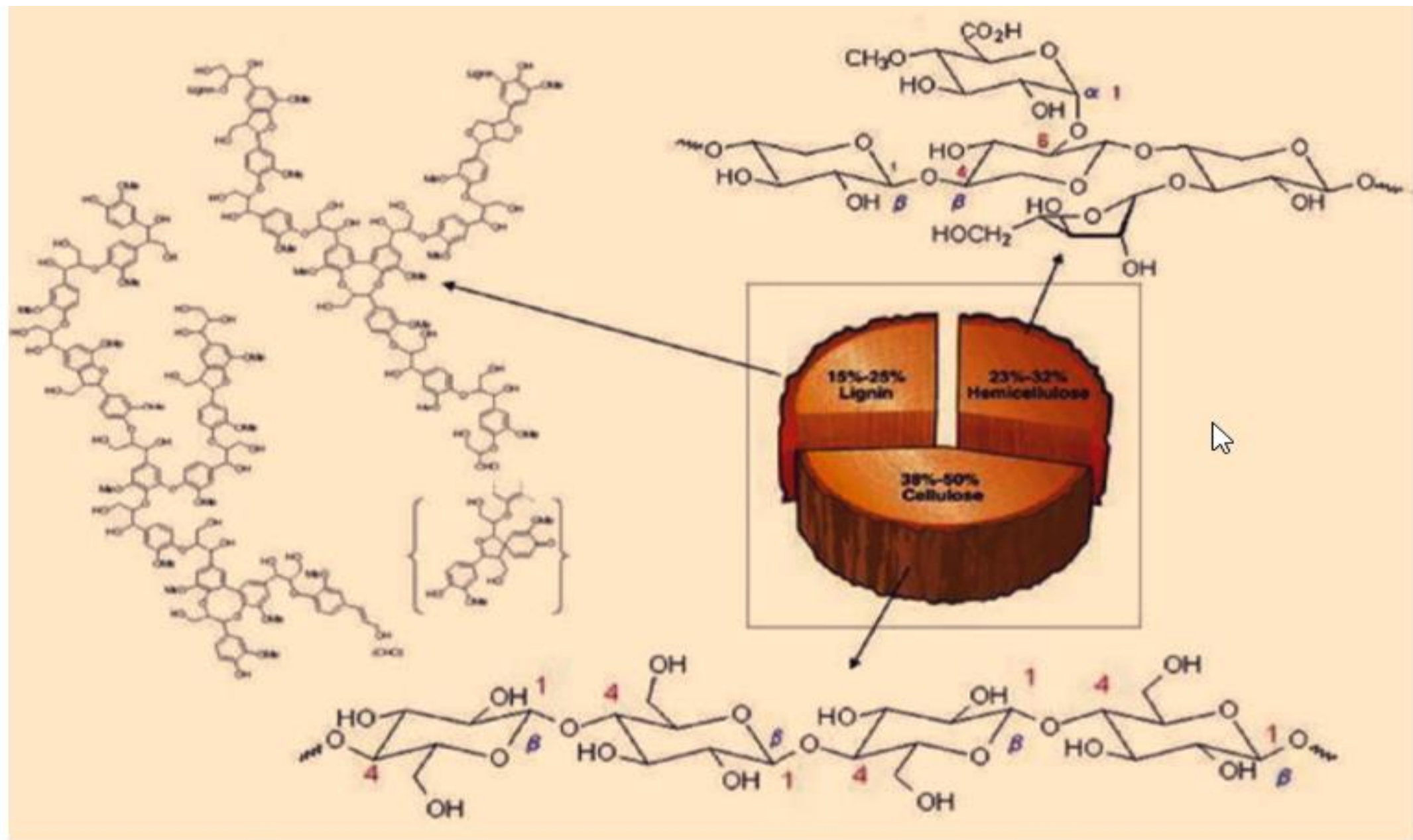
- **Lignin**

While cellulose is regarded as the most widely abundant natural polymer and indeed the most abundant natural resource, lignin is the most abundant aromatic polymer in nature and the next most abundant polymer. Lignin comes from the Latin word **lignum**, meaning wood. Lignin was first referred to as a constituent of wood *by Ansleme Payen* in 1838 as the carbon substance acting as the matrix in wood composite embedding cellulose in wood. Later in 1865 this matrix was identified as lignin *by Schulze*.

- **Lignins**

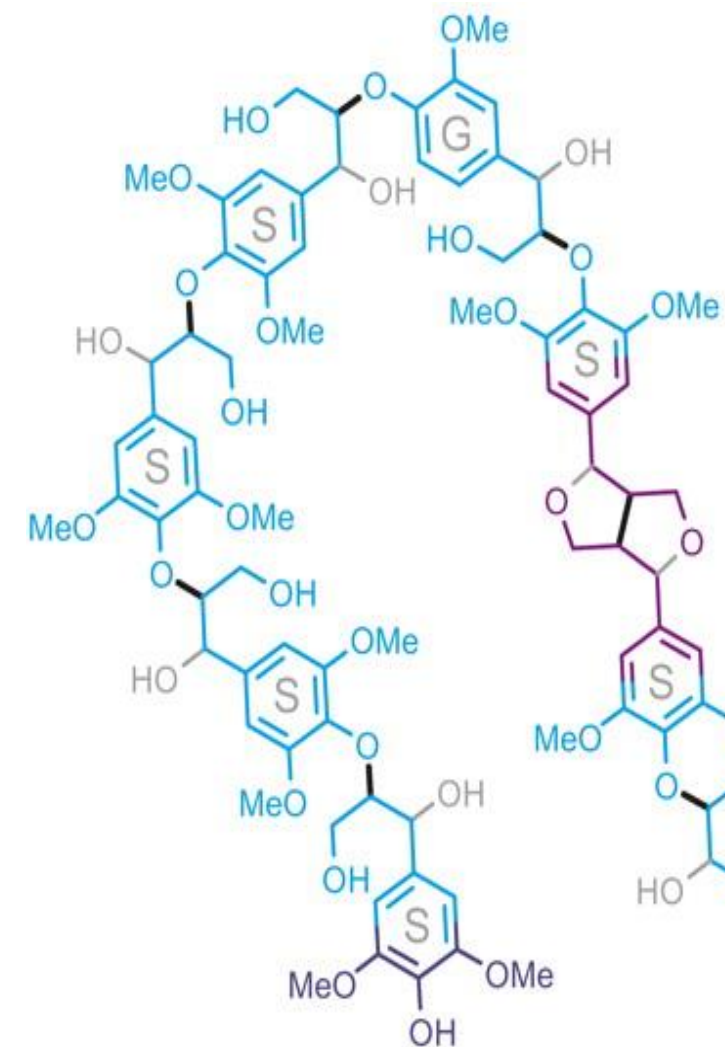
are heteropolymers with rather complex structures. These hydrophobic polymers exist in plant cell walls providing the matrix that binds the cellulose microfibrils and other components of the cell walls, thus providing biomechanical strength and rigidity. They are responsible for the upright growth of plants.

- **Lignin** is formed within the spaces existing around the cellulose microfibrils in the final stage of cell differentiation in plant cell walls, thus forming a lignocelluloses matrix which contributes to the strength of the plant.
- **Lignin** is generally viewed as a waste material from industrial processes such as pulp and paper production and ethanol production from lignocelluloses biomass. It makes up about 20–30% of cellulosic biomass. It is considered as non-fermentable, however, it is useful as a boiler fuel. On average, between 40 and 50 million tons of lignin is produced as a waste by-product from the pulp and paper industry.

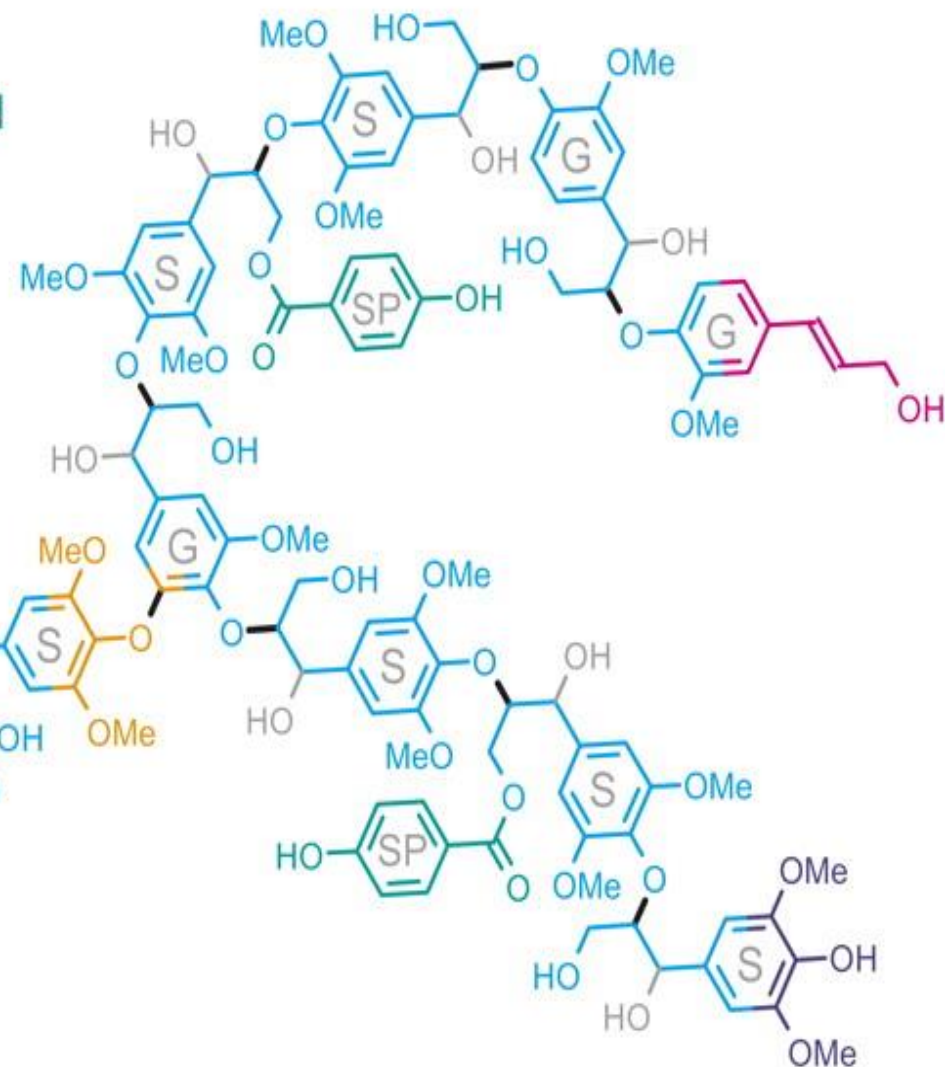


Structure of lignin and other wood constituents (Sourced from Adler (1977) with

- The **precise structure** of native lignin is yet to be known and the structure of a particular lignin varies with source and extraction method. However, it is known that it contains mainly methoxyl groups, phenolic hydroxyl groups, and a few thermal aldehyde groups approximately in the following proportions:
- **carbonyl** 10–15 %, **benzyl alcohol** 15–20 %, **phenolic hydroxyl** 15–30 %, and **methoxyl** 92–96 %



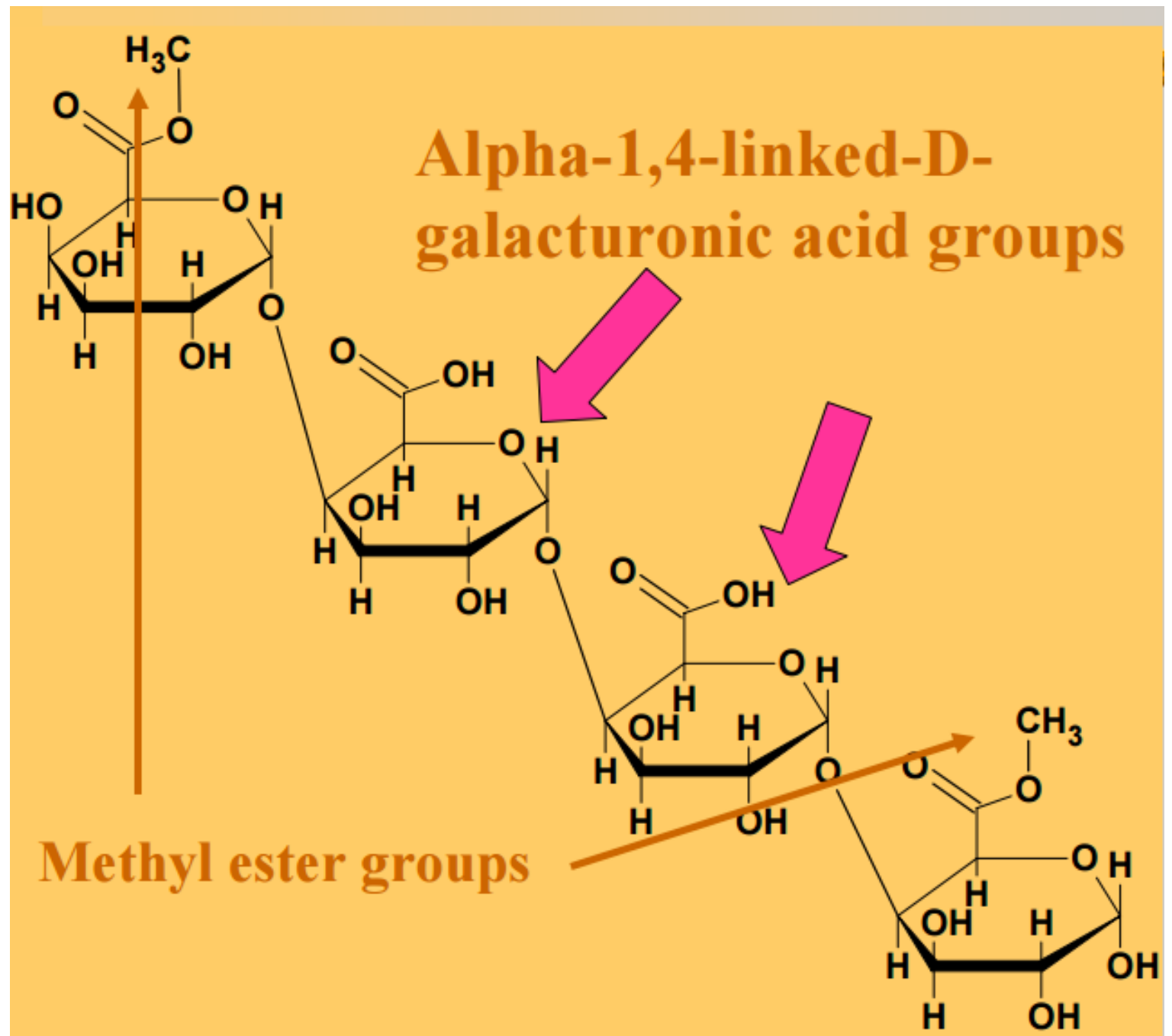
S syringyl
 G guaiacyl
 SP sinapyl *p*-hydroxybenzoate-derived
 β -O-4, β -aryl ether
 β -5, phenylcoumaran
 β - β , resinol
 4-O-5, biphenylether
 cinnamyl alcohol endgroup
 phenolic endgroup



- **Pectin**

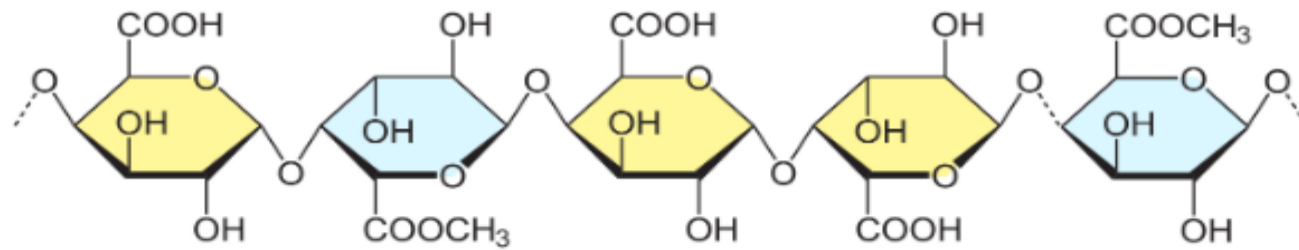
- Pectin refers to a complex group of molecules with a framework of mainly α -D-(1-4)_galacturonan with intermittent units of α -L-(1-2)rhamnose. It belongs to the class of gel forming polysaccharides alongside others such as agar and mucopolysaccharides.
- **Pectin** is present in the primary cell wall of plants alongside other components such as cellulose, hemicelluloses, and lignin. It makes up to 35 % of the dry weight of the cell walls of dicotyledon higher plants. It constitutes less proportion and is of different forms in monocotyledon plants.

- Pectin acts as a structural and developmental polysaccharide in plants and also contributes its ion exchange capacity, thus regulating the movement of ions and the pH of the plant cell wall.

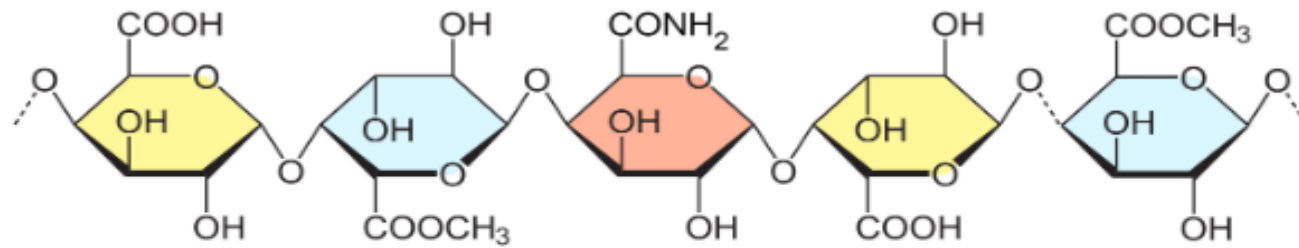


LM pectins

Modification of the extraction process, or continued acid treatment, will yield conventional low methoxyl (LMC) pectin with less than 50% methoxyl groups.



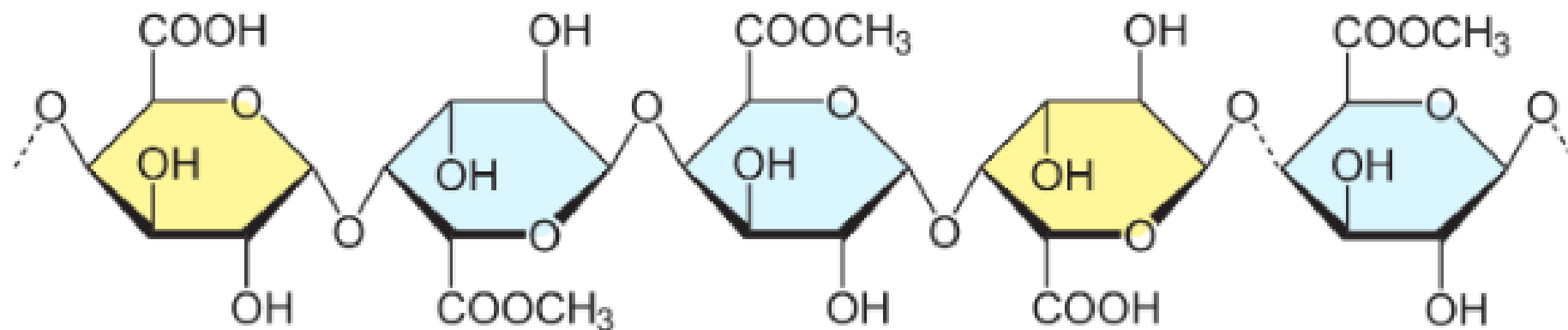
LMC PECTIN



LMA PECTIN

HM pectins

Pectin produced by the normal extraction process contains more than 50% of methoxyl groups and is classified as high methoxyl (HM) pectin. They are capable of forming gels in aqueous systems with high contents of soluble solids and low pH values.



HM PECTIN

The following classification is based on the average pectin content in different fresh fruits:

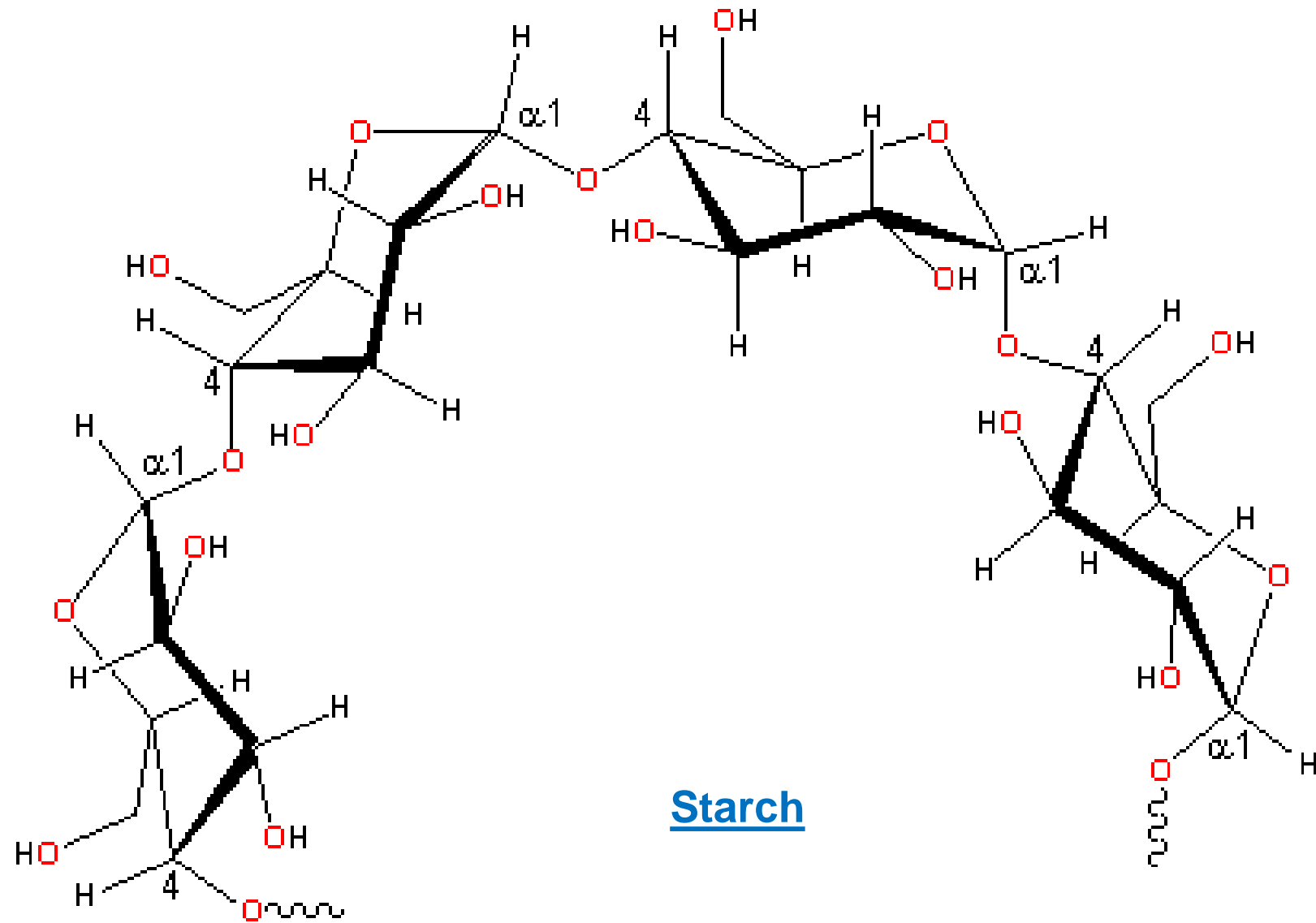
- High pectin content: lime, lemon, orange, and apple
- Average pectin content: apricot and blackberry
- Low pectin content: cherry, peach, and pineapple

In the citrus fruits (orange, lemon, and lime), pectin is mainly located in the albedo, the inner white layer of the peel that surrounds the juicy vesicles, and the lamellae. When the juice is extracted from the fruit, the peel and the albedo can be used to make the pectin.



- ***Starch (Amylose and Amylopectin)***

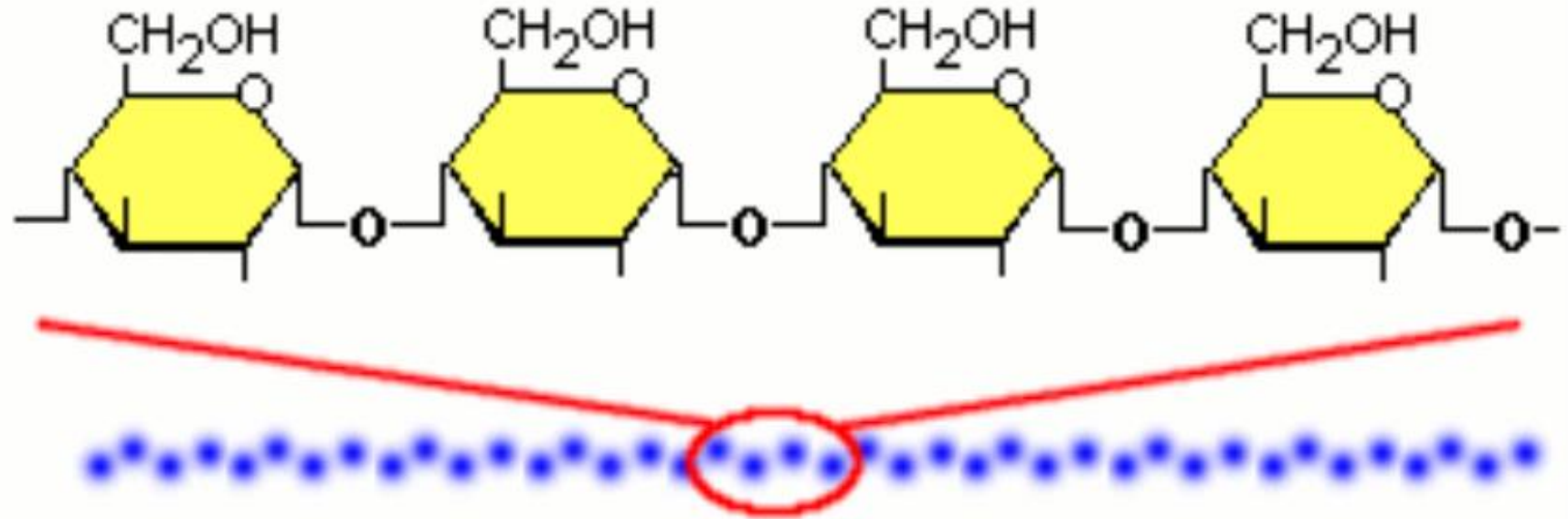
- Starch in its pure form is an odorless, tasteless white powder. It is a polysaccharide that consists of two types of molecules; amylose and amylopectin. The concentration of each varies with the source and type of starch, however, it is usually around 20–25 % w/w amylose and 75–80 % amylopectin.
- **Starch is generally insoluble in water and alcohol; however, in the presence of heat and water it can be irreversibly dissolved in water** by the process known as gelatinization. Starch is used in a variety of industrial applications, mainly adhesives, paper, and clothing. The application of starch for various purposes dates as far back as 700 A.D. when it was applied as cosmetic creams, food thickener, and in paper production.



Starch

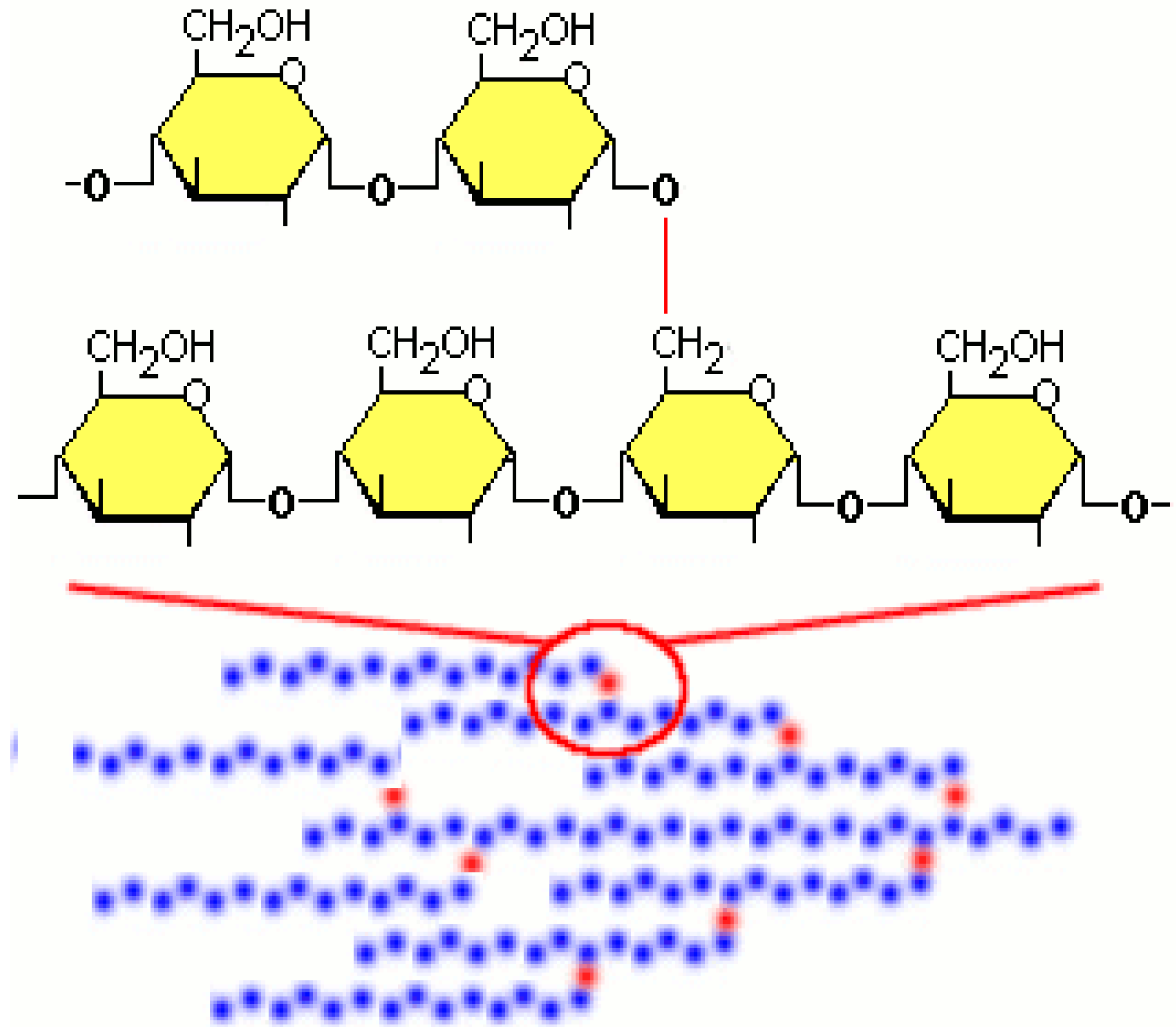
Amylose is a water soluble polysaccharide made up of (1-4)- α -d linked polyglucan in a wobbled helix configuration, while amylopectin takes a branched form with branching occurring after every 28–30 glucose unit.

The branched configuration of amylopectin relative to amylose makes it more susceptible to hydrolysis and degradation as it has more regions exposed.



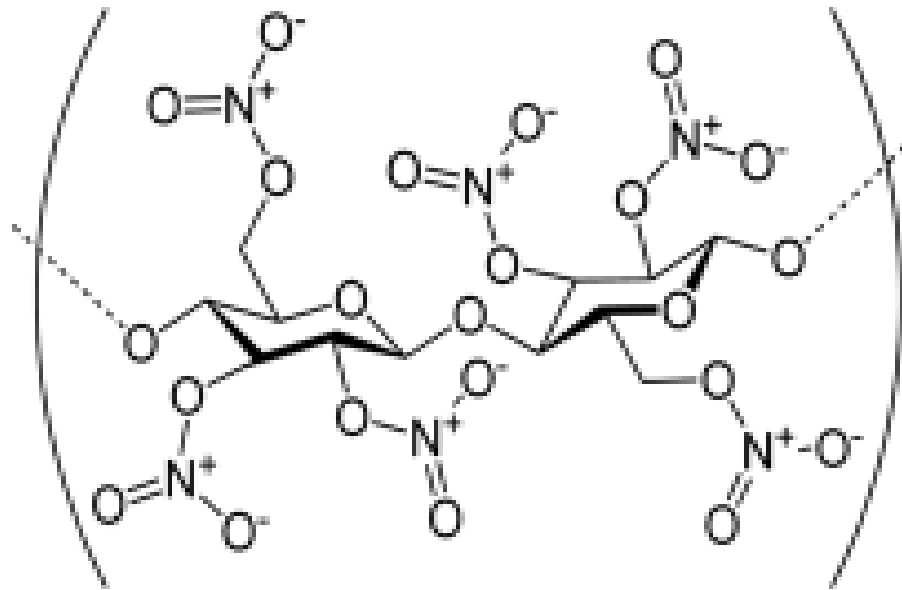
Amylose consists of a linear, helical chains of roughly 500 to 20,000 alpha-D-glucose monomers linked together through alpha (1-4) glycosidic bonds.

Amylopectin molecules are huge, branched polymers of glucose, each containing between one and two million residues. In contrast to amylose, amylopectin is branched. It contains numerous amylose-like chains of up to 30 glucose residues linked through **alpha (1-4) bonds**, connected to one another through **alpha (1-6) branch points**.

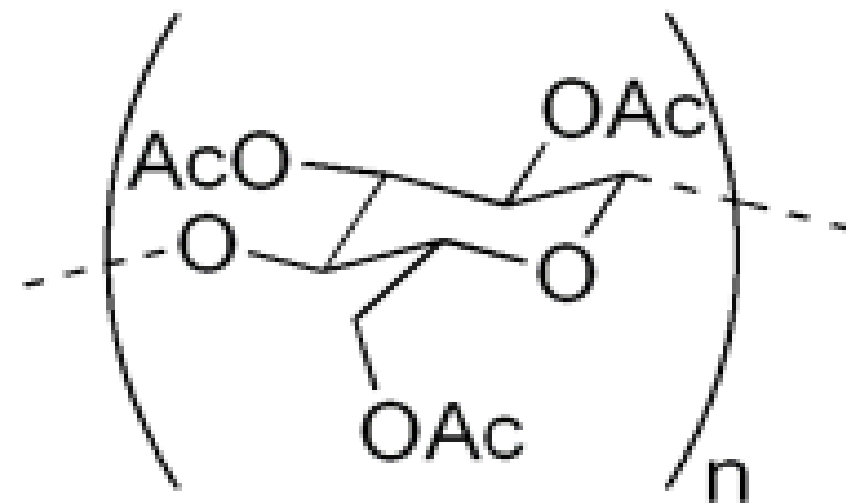


Semi-synthetic Polymers: Cellulose nitrate and cellulose acetate

obtained from natural polymers by subjecting them to some chemical processes



Cellulose nitrate



Cellulose triacetate



The TGA curve, Fig. 1, shows that cellulose triacetate (CTA) degraded in three steps. The first step from the room temperature (25°C) to 330°C, represents the **volatilization of the volatile matter**, and/or the evaporation of residual absorbed water.

The second step starts at 330°C and ends at 500°C, and represents the **main thermal degradation of the cellulose acetate** chains.

The third step starts at 500°C, and represents the **carbonization of the products to ash..**

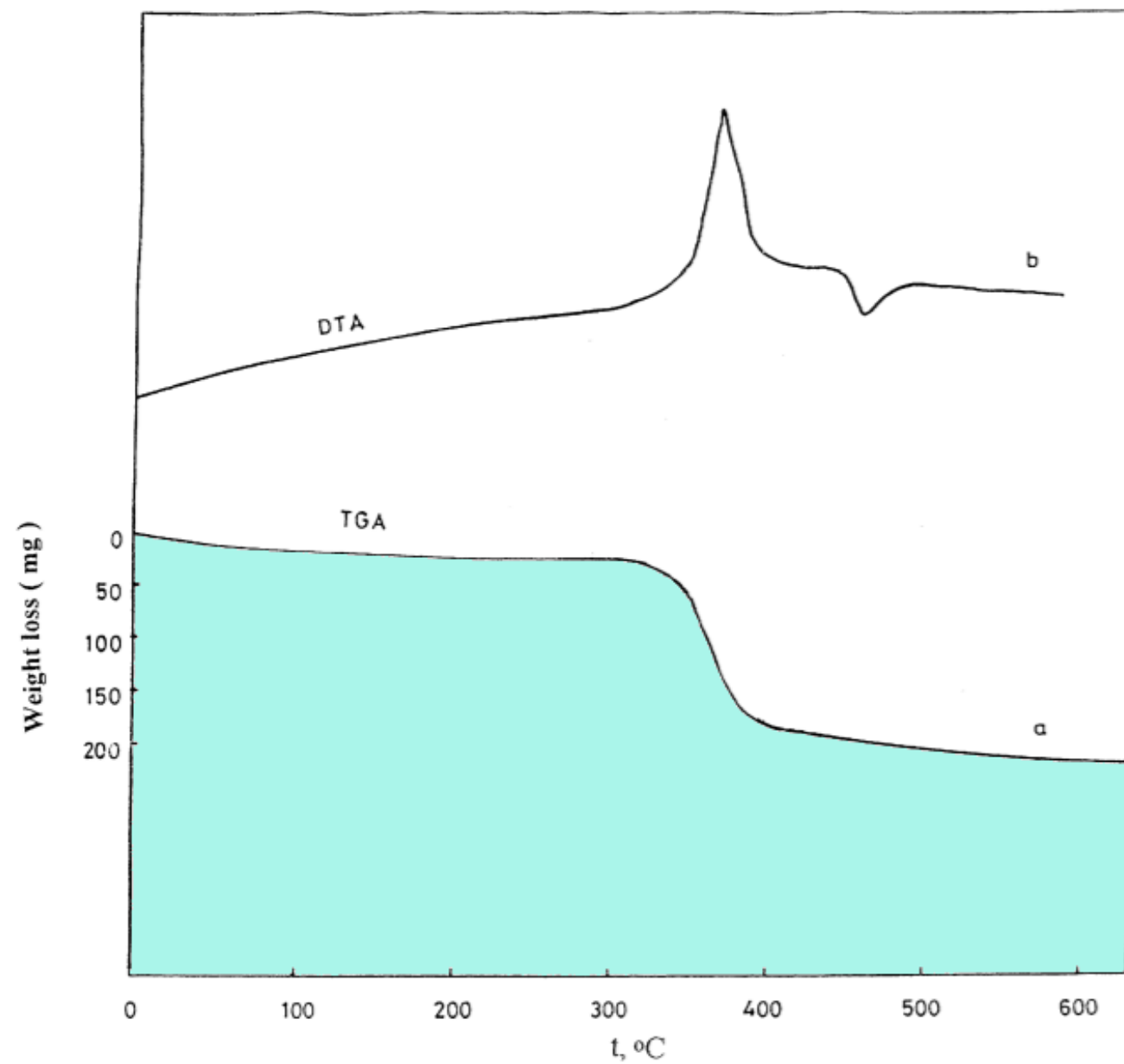


Fig. 1. TGA and DTA curves of CTA.

Rapid Production of a Porous Cellulose Acetate Membrane for Water Filtration using Readily Available Chemicals

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- A procedure for the production of **mesoporous microfiltration membranes** by removing a nanoparticle pore template was developed by **Kellenberger** et al. In their method, calcium carbonate nanoparticles were used as pore templates to fabricate polymeric membranes with tunable pore sizes. This technique was up scaled to an industrial level and resulted in the development of the commercially available **Drink Pure water filter**. The key part of this water filter is a microfiltration membrane that is produced via pore tempelation using calcium carbonate nanoparticles

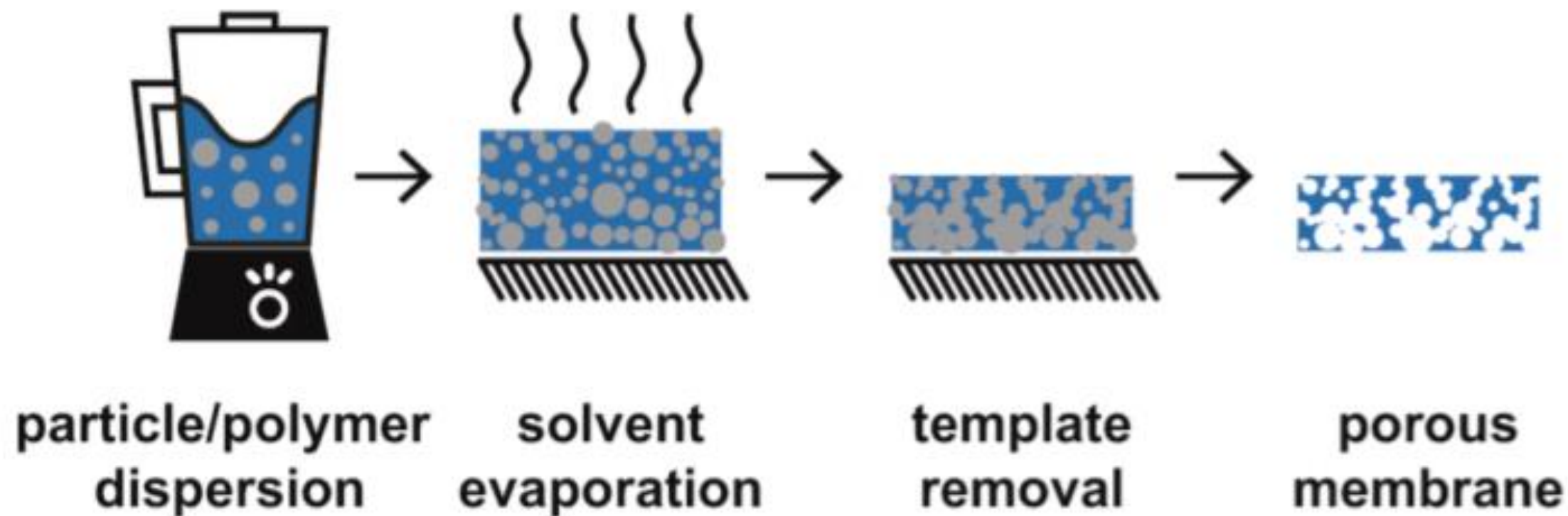


Figure 1. Production of polymeric membranes using a kitchen blender. A calcium carbonate particle–polymer dispersion is cast on a glass support and the solvent evaporated in ambient air. Acid bath incubation of the membrane dissolves the calcium carbonate particles and yields a porous membrane.

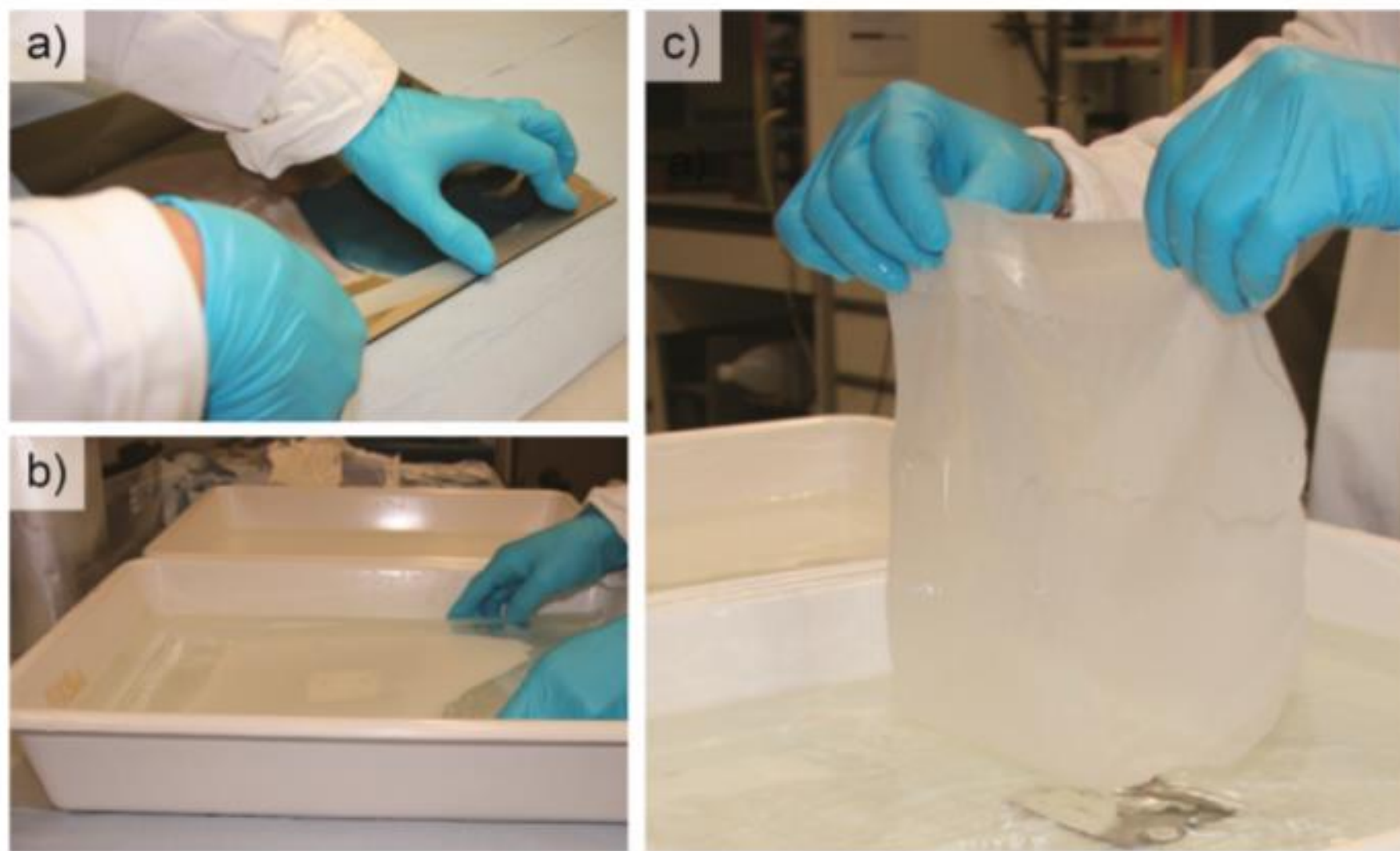


Figure 2. (a) Preparation of mirror plate for dispersion-casting, (b) acid bath incubation of cellulose acetate membrane, and (c) final product, removed from the washing bath.

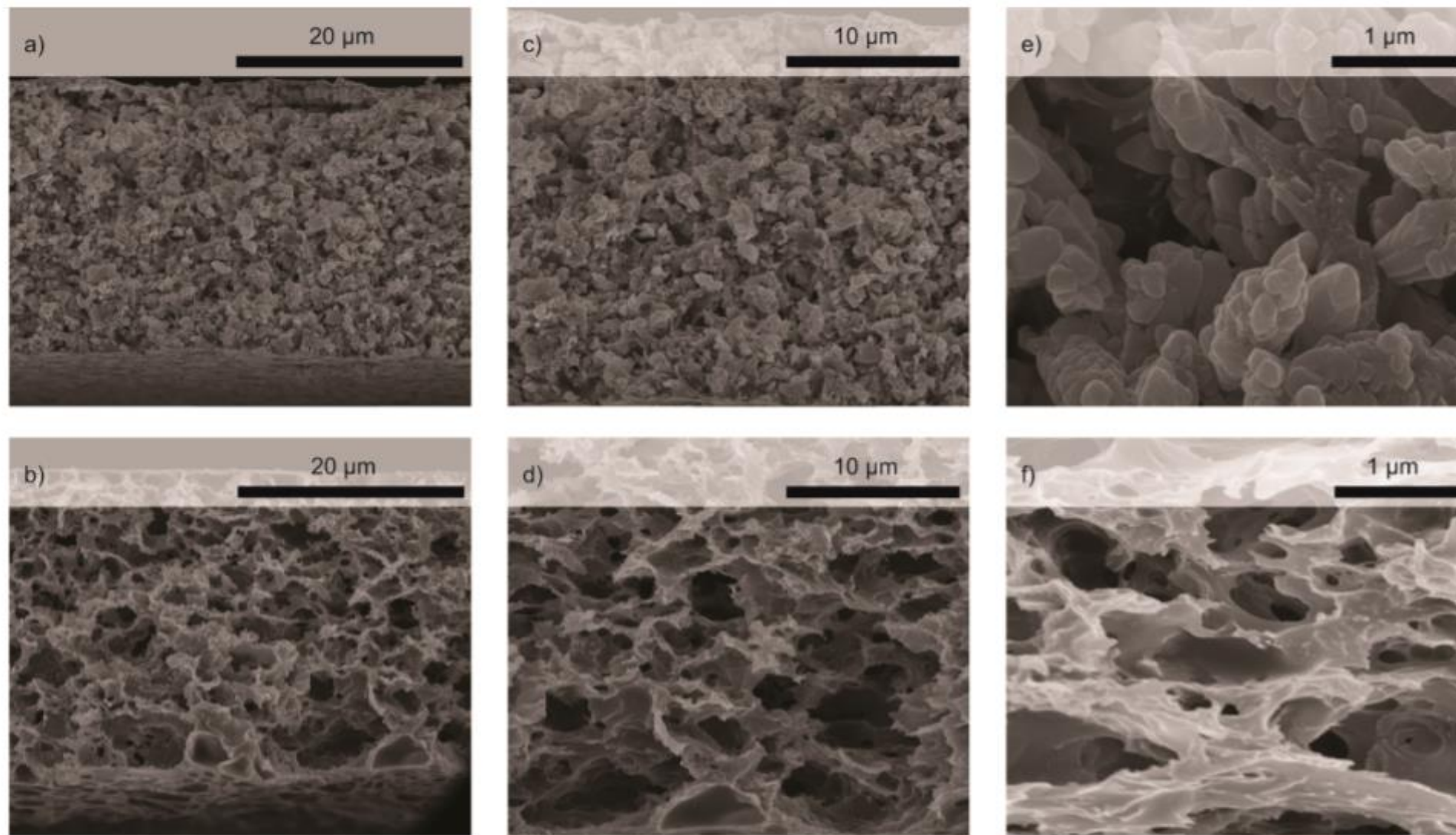


Figure 3. Scanning electron microscopy (FEI nova NanoSEM 450, 3 kV, spot size 2.5) images of cellulose acetate membrane cross sections at various magnifications. Images on the top row show the membrane cross sections prior to removal of the calcium carbonate pore template; images on the bottom row show the membrane cross sections after the calcium carbonate pore template has been removed.

Physical Properties of Cellulose Acetate Rayon: Acetate rayon is a soft fiber and it has not enough elasticity as other **manmade fiber**. Anyhow, followings are the physical properties of cellulose acetate rayon.

1. **Tenacity:** 0.9 – 1.4 gm/den
2. **Density:** 1.32 gm/c.c
3. **Elongation at break:** Very good
4. **Elasticity:** Not so good
5. **Moisture Regain (MR%):** 6.0%
6. **Resiliency:** *Not good*
7. **Melting point:** 230⁰C
8. **Ability to protest friction:** Moderate
9. **Color:** White or color less
10. **Light reflection ability:** It can protect up to 275⁰F temperature.
11. **Lusture:** light to bright

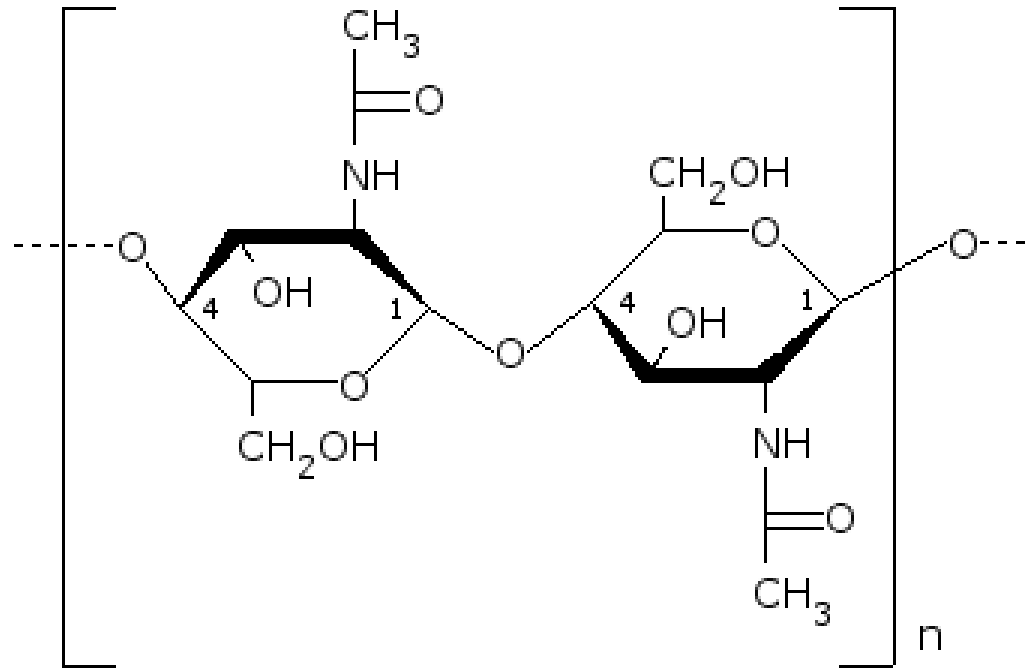
Chemical Properties of Cellulose Acetate Rayon: Various types of chemical properties of Cellulose Acetate Rayon fiber are given below:

1. **Acids:** It becomes soluble when immersed in acetic acid solution. Strong acids are harmful for this fiber.
2. **Basic:** Strong alkali damages the cellulose acetate fiber greatly. It also slightly damaged by weak alkalis.
3. **Effect of bleaching:** It damaged by strong oxidizing agents. But it has enough protection ability against weak oxidizing and reducing agents.
4. **Organic solvent:** It soluble in acetone. Dry cleaning agent does not affect the cellulose acetate rayon.
5. **Protection ability against mildew:** Mildew does not damage the cellulose acetate rayon.
6. **Protection ability against insects:** Insects do not cause harm to the cellulose acetate rayon.
7. **Dyes:** Special types of dyes are required.

Chitin



the exoskeletons of crabs,
lobsters and shrimps

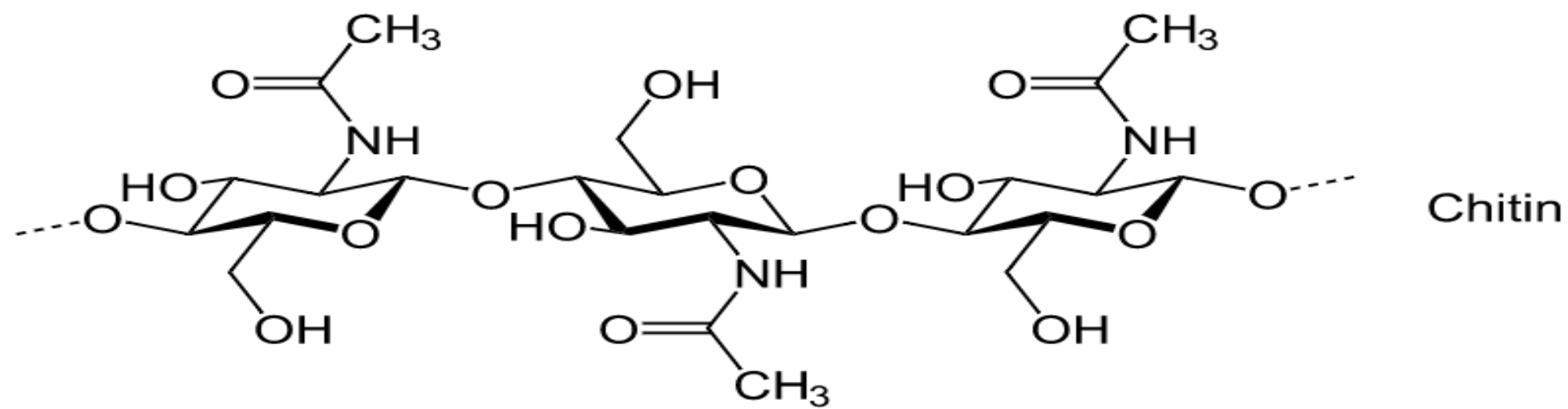


(polymer of the N-Acetylglucosamine units)

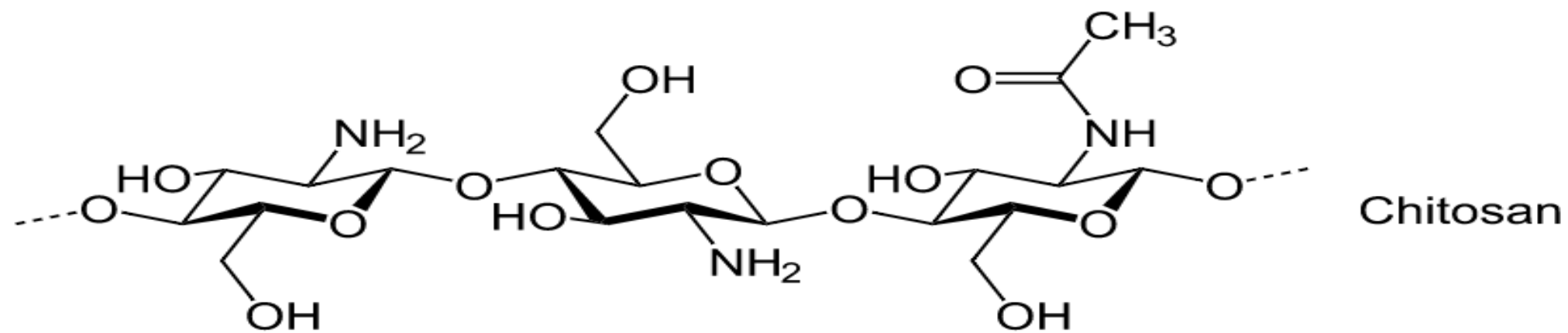
Chitin is a highly hydrophobic linear polysaccharide of animal origin containing amino and acetyl groups within its unit. It is insoluble in water and other organic solvent, it is however soluble in specialized solvents such as chloroalcohols in conjugation with aqueous solutions of mineral acids, hexafluoro-isopropanol, and dimethylacetamide containing 5 % lithium chloride.

- It is another abundantly available structural polysaccharide in nature, present in the exoskeleton of invertebrates such as insects, crustaceans, and other organisms including in the mycelia and spore of fungi .
- **Chitin** is similar in structure to cellulose except for the hydroxyl groups contained within the cellulose chain that are substituted with an acetamido group .

- Unlike most natural polymers , **chitin (as well as chitosan)** is alkali in nature. This gives it some desirable properties for various applications such as film and gel forming ability, chelation of metal ions, and formation of polyoxysalts.
- **Chitin** is especially applied in biotechnology in the modified form of chitosan which is obtained from the deacetylation of chitin.
- **Chitosan** is widely applied in **transdermal drug delivery**,
- particularly for its mucoadhesive, reactive, and mechanical property, for its tendency to be insoluble in neutral and alkali environment, and its solubility in acidic environment which makes it attractive in controlled delivery.



↓ Chitin-Deacetylase





The **chitosan filled thermoplastic elastomer (TPE)** composites with **different filler loading** was prepared by melt mixing at 180 °C. The effect of 3-aminopropyltriethoxysilane (3-APE) as coupling agent on thermal properties of composites were investigated. The melt flow indexer was used to characterize the melt flow index (MFI) of TPE/Chitosan composites at temperature of 180 to 210 °C. The TGA results reported that the composites had better thermal stability and lower total weight loss as compared to untreated composites at similar filler loading.

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Filler Treatment. The 3-APE delivered was in liquid form. The 3 % of 3-APE was dissolved in ethanol (v/v). Subsequently, chitosan powder was added into the solution and stirred with constant speed for 1 hour. The treated chitosan was dried in oven at 80 °C for 24 hours to evaporate the ethanol completely.

The TPE used was Hostacom CA199AC, which was supplied by Poly Pacific Polymers Sdn. Bhd., Port Klang, Malaysia. It was in pellet form with density of 0.89 g/cm³

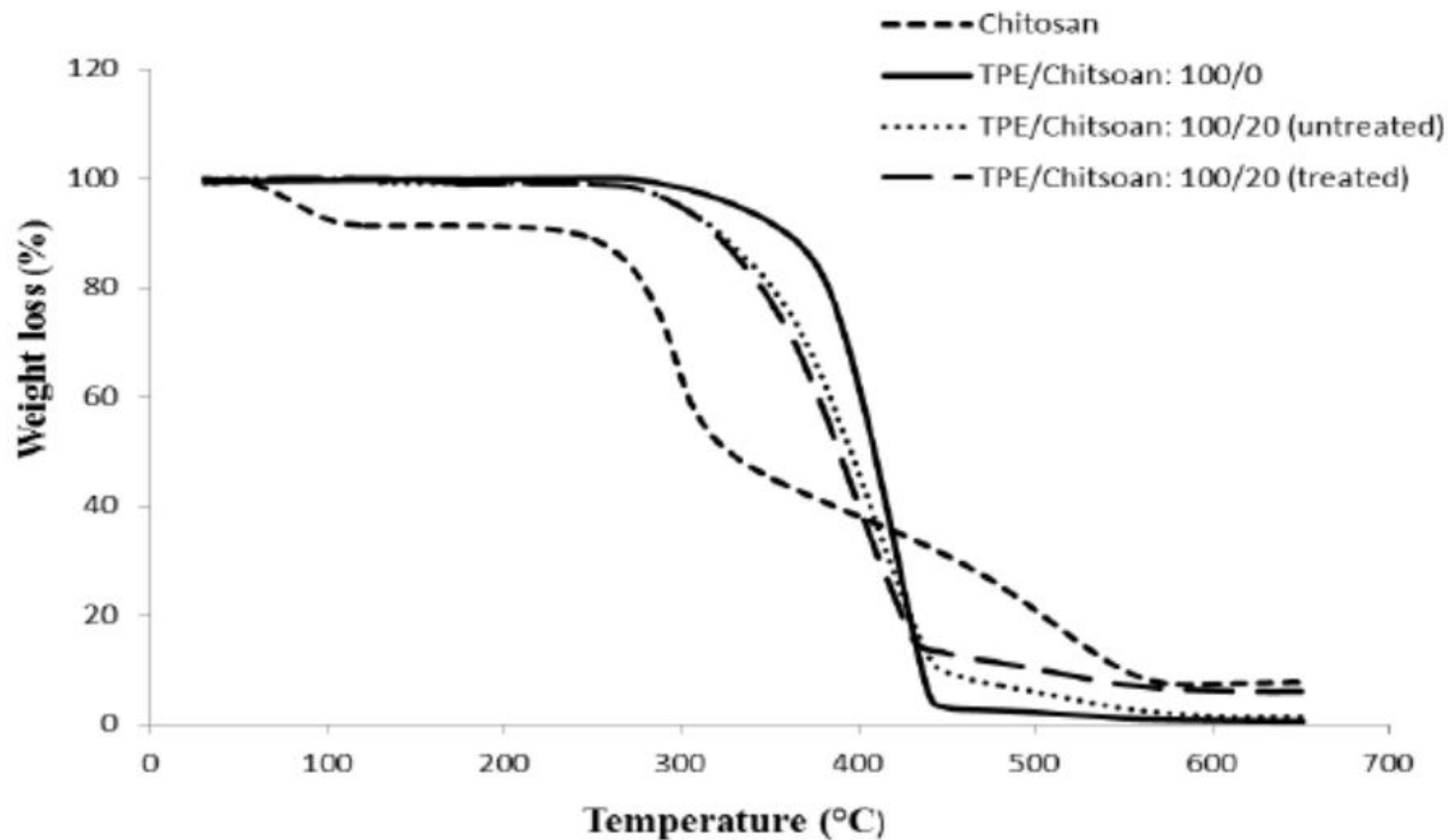
Its melt flow index was 2.5 g/10 min at 230 °C with 2.16 kg load.

Composites Preparation. Composites were prepared by using a Z-Blade mixer at temperature of 180 °C and rotor speed of 50 rpm. The TPE were loaded into the mixing chamber for 5 minutes. After 5 minutes, filler was added and mixing continued for 10 minutes. The total mixing time of composites was 15 minutes. Finally, the composites were removed from the mixing chamber and pressed into thick round pieces. The same procedure was repeated by replaced chitosan with the 3-APE treated chitosan.

Table 1. Formulation for TPE/chitosan composites

Materials	Untreated composites	Treated composites
TPE [php]	100	100
Chitosan [php]	0, 10, 20, 30, 40	10, 20, 30, 40
3-APE [%]	-	3*

* 3 % based on weight of chitosan



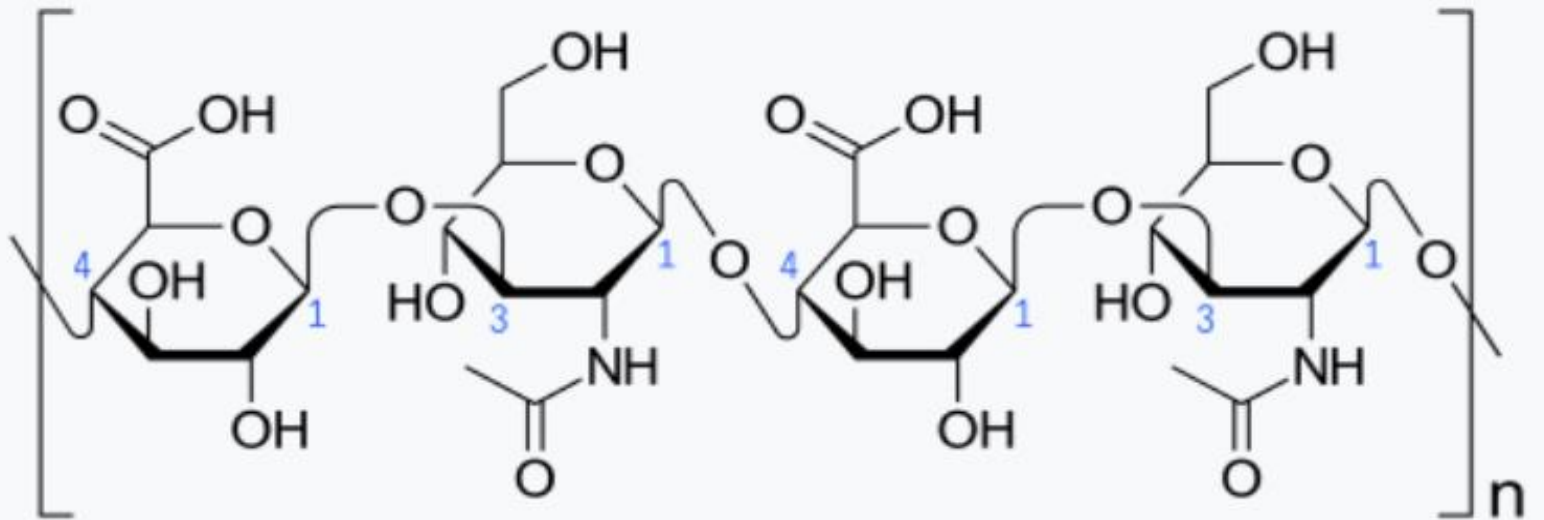
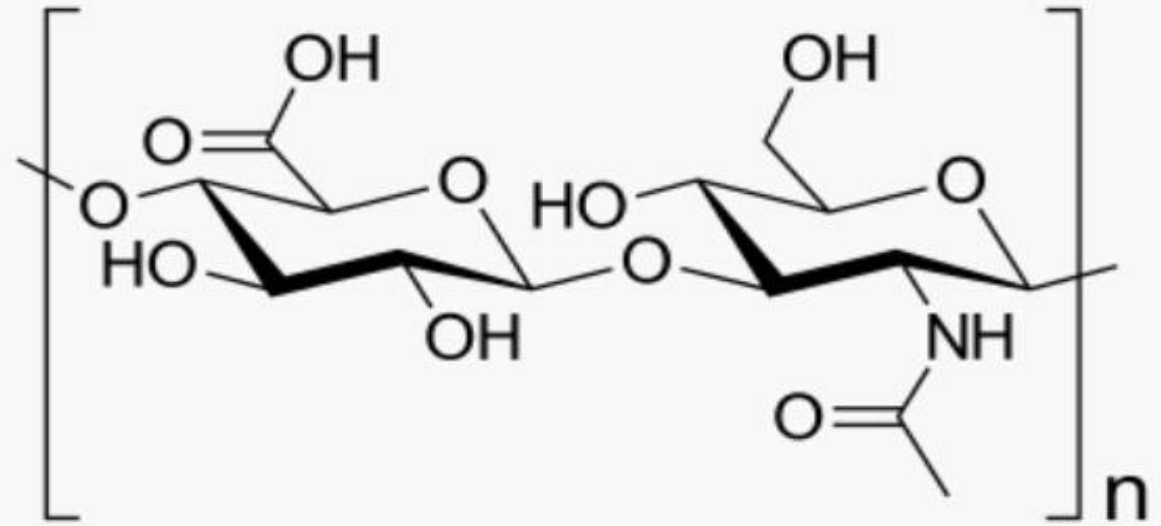
Comparison of thermogravimetric analysis of chitosan, untreated and treated TPE/Chitosan composites.

- ***Hyaluronic Acid***
- Hyaluronic acid is an example of a **glycoprotein**, also known as **mucopolysaccharides** or mucins.
- It is also one of the few polysaccharides found in the tissue of vertebrates and more abundantly in young embryo; another example of such is heparin.
- These are polysaccharides bound to proteins in a covalent bond. Other types include proteoglycans.

- **Hyaluronic acid or hyaluronan** that plays a significant role in tissue development and cell proliferation is a straight chain polysaccharide with a molecular mass of about 7×10^6 g/mol, usually strongly attached to proteins in a hydrogen bond similar to that of water, thus making its extraction and isolation rather onerous. Nevertheless, pure hyaluronic acid has been isolated from sources such as cord bovine vitreous humor and rooster combs and bacteria streptococcus zooepidemicus

Hyaluronic acid

It is useful for certain biomedical applications; for instance, in combination with alginate it is used in surgical applications for **wound healing**.



Haworth projection

- **Hyaluronic acid** is an important component of articular cartilage, where it is present as a coat around each cell (chondrocyte). When aggrecan monomers bind to hyaluronan in the presence of HAPLN1 (hyaluronan link protein 1), large, highly negatively charged aggregates form. These aggregates imbibe water and are responsible for the resilience of cartilage (its resistance to compression). The molecular weight of hyaluronan in cartilage decreases with age, but the amount increases.

- A **lubricating role of hyaluronan** in muscular connective tissues to enhance the sliding between adjacent tissue layers has been suggested. A particular type of fibroblasts, embedded in dense fascial tissues, has been proposed as being cells specialized for the biosynthesis of the hyaluronan-rich matrix. Their related activity could be involved in regulating the sliding ability between adjacent muscular connective tissues.

- ***Alginate***

- Alginate is a long chain **hydrophilic polymer** sourced from seaweeds, where it exists within the cell walls providing flexibility and strength.
- It has been in use as food as far back as 600 B.C.
However, it was not until 1896 that the purified form of alginate was extracted from seaweed by Akrefting.



Algae are a diverse group of aquatic organisms that have the ability to conduct photosynthesis. Certain **algae** are familiar to most people; for instance, seaweeds (such as kelp or phytoplankton), pond scum or the **algal** blooms in lakes. Jun 4, 2016

- It usually exists in association with other cations, mainly sodium and calcium as sodium alginate and calcium alginate. The cations attached to the alginate have an effect on its properties. The properties of the alginate also depend on the species of algae, which is mainly *Laminaria hyperborean*, *Macrocystis pyrifera*, *Laminaria digitat*, and *Ascophyllum nodosum*. Bacteria of the species *Pseudomonas* and *Azotobacter* also produce alginate-like polymeric materials.

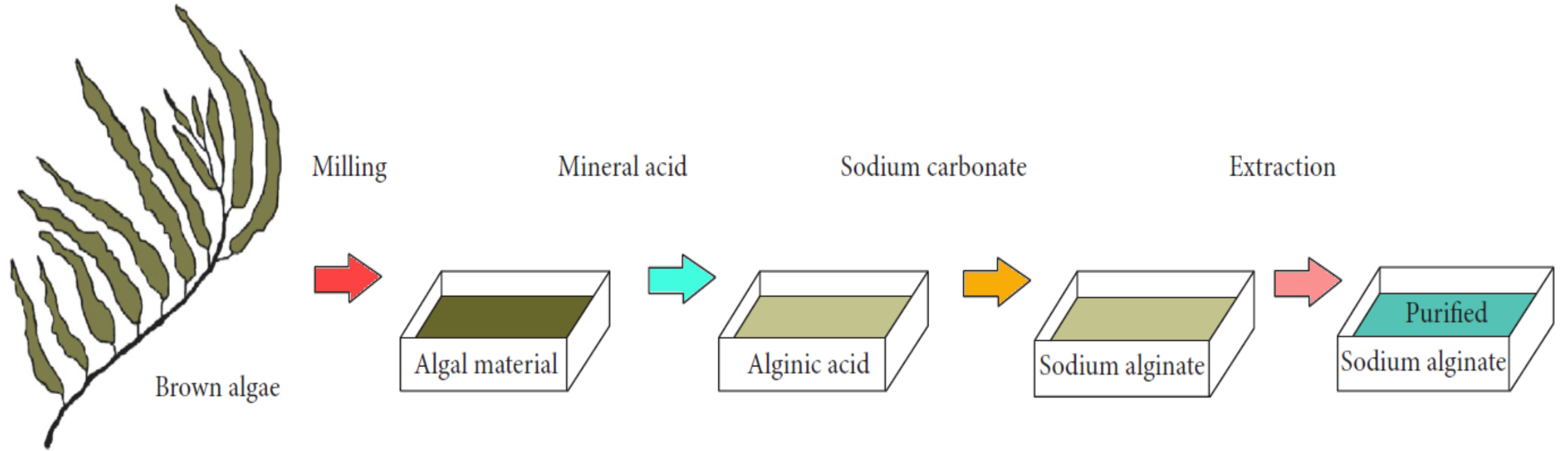
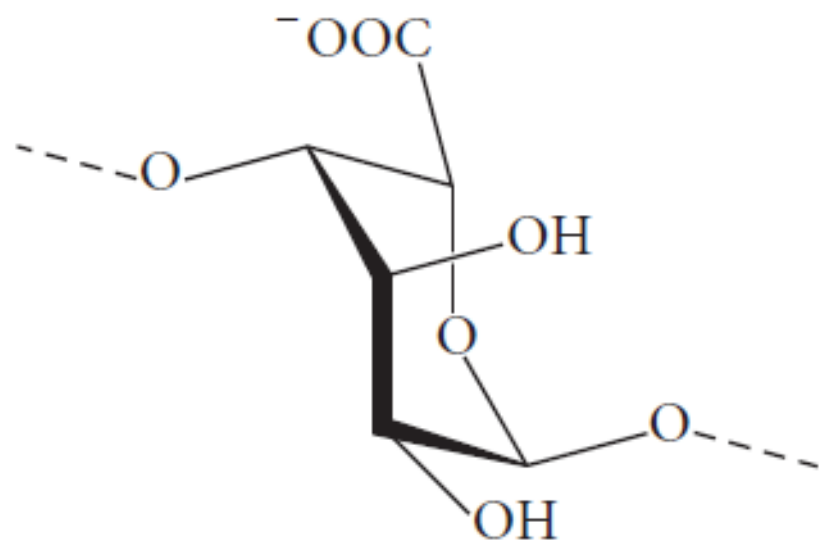
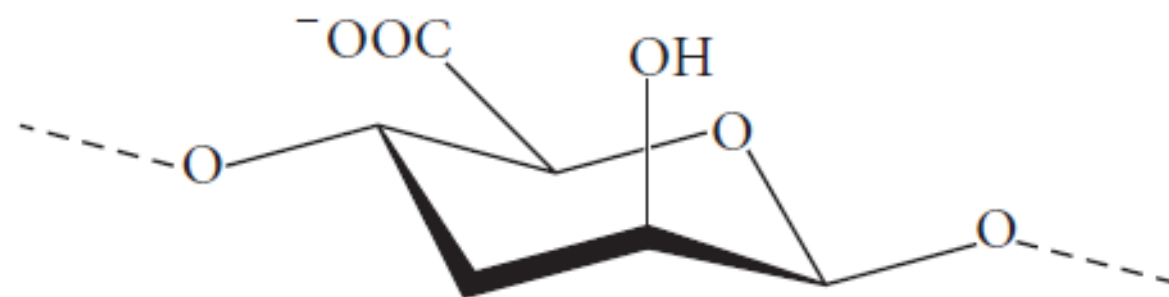


FIGURE 2: The procedure of sodium alginate extraction from brown algae [2].

- **Alginic acid** serves diverse biological functions and has various industrial applications as a **stabilizing agent**, **drug carrier**, **viscos**, and as **binding agent**.
- It is also used in combination with other polymers such **as chitosan and hyaluronic acid** to serve more varied functions.



1,4 α -L-Guluronic acid



1,4 β -D-Mannuronic acid

(a)

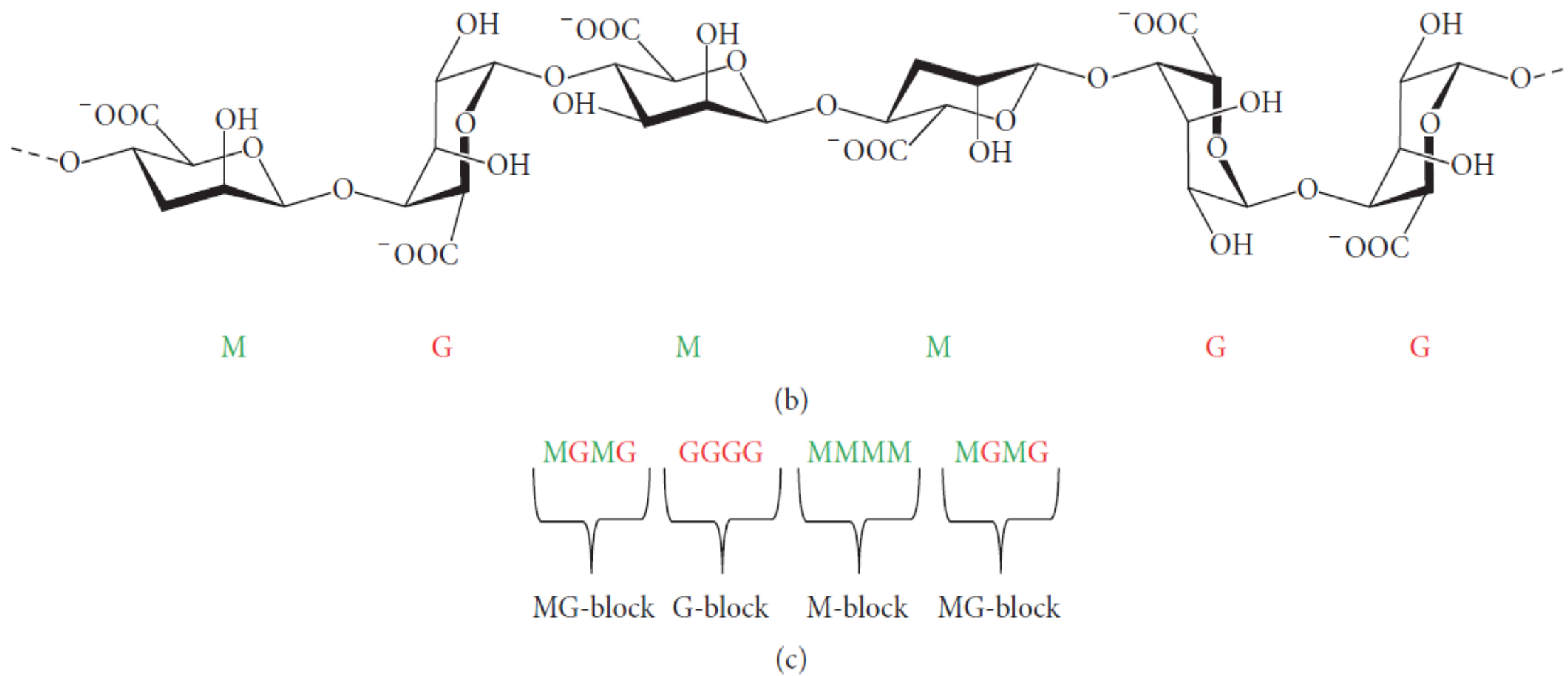


FIGURE 1: The structure of ALG: monomers (a), chain conformation (b), and blocks distribution (c).

- **ALG are commercially available** in various grades of molecular weight, composition, and distribution pattern of **M-block and G-block**, the factors responsible for their physicochemical properties such as viscosity, sol/gel transition, and water-uptake ability.
- The molecular weight, expressed as an average of all the molecules present in the sample, of commercial ALG varies between 33 000 and 400 000 g/mol. ALG extracted from different sources differ in M and G residues as well as the length of each block. Generally, by raising the ALG G-block content or molecular weight, more stronger and brittle ALG gels may be achieved

ALG can be easily formed into diverse semisolid or solid structures under mild conditions because of their unique ability of sol/gel transition. Therefore, ALG are commonly used as viscosity increasing agents, thickeners, and suspension and emulsion stabilizers in food and pharmaceutical industry .

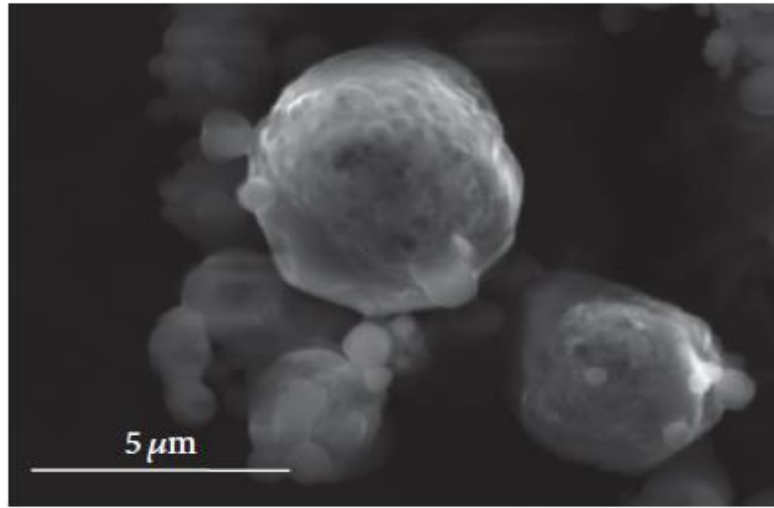
Alginic acid is insoluble in water and organic solvents, whereas ALG monovalent salts and ALG esters are water-soluble forming stable, viscous solutions

The 1% w/v aqueous solution **of sodium alginate** has a dynamic viscosity 20–400mPa·s at 20°C. ALG solubility is limited by the solvent pH (a decrease in pH below pKa 3.38–3.65 may lead to polymer precipitation), ionic strength, and the content of “gelling ions”

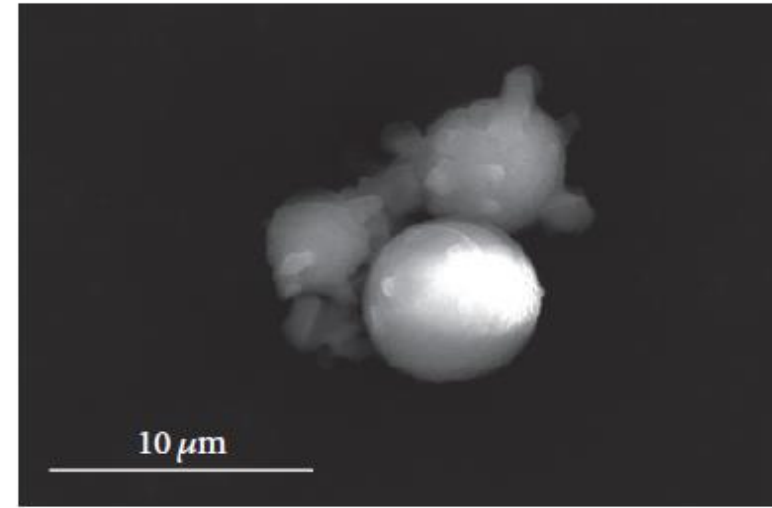
ALG gelation can be induced in the presence of divalent ions, which cross-link the polymer chains through the “eggbox” model or by lowering the pH value below the pKa of ALG monomers using lactones like d-glucono- δ -lactone. It should be noted that calcium chloride, most frequently used source of Ca^{2+} ions, is responsible for rapid and uncontrollable ALG gelation.

Due to large surface area, which may favour an intimate contact between the polymer and mucin, multiunit dosage forms with sodium alginate are also explored as **gastro retentive drug carriers** (Figure 4), especially for substances, which are unstable or degraded in the alkaline pH.

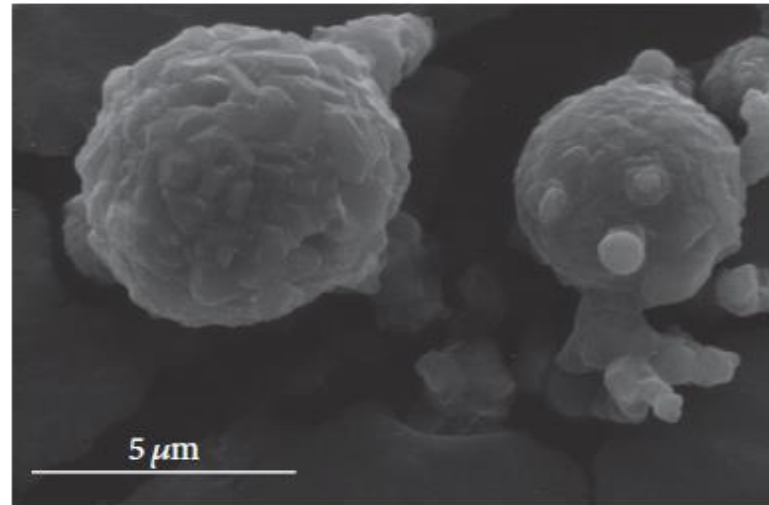
ALG have been extensively evaluated as **vaccine adjuvants** or coadjuvants as these polymers were displayed to enhance bioavailability and immunogenicity of antigens after nasal and oral administration



(a)



(b)



(c)

FIGURE 4: SEM images of alginate microspheres obtained by the spray drying method with metronidazole under magnification $\times 20\,000$ (a), ranitidine hydrochloride under magnification $\times 10\,000$ (b), and metformin hydrochloride under magnification $\times 20\,000$ (c) (author's unpublished images).

The **TG curves** presented in Figure 2 suggest that, under heating, the alginic acid presents initially a dehydration process followed by decomposition in two overlapping steps under nitrogen (Figure 2a), without residue at the end of the experiment. Similar behavior was observed in air (Figure 2b). The decomposition product around 400 °C was characterized as a carbonaceous material in both cases.

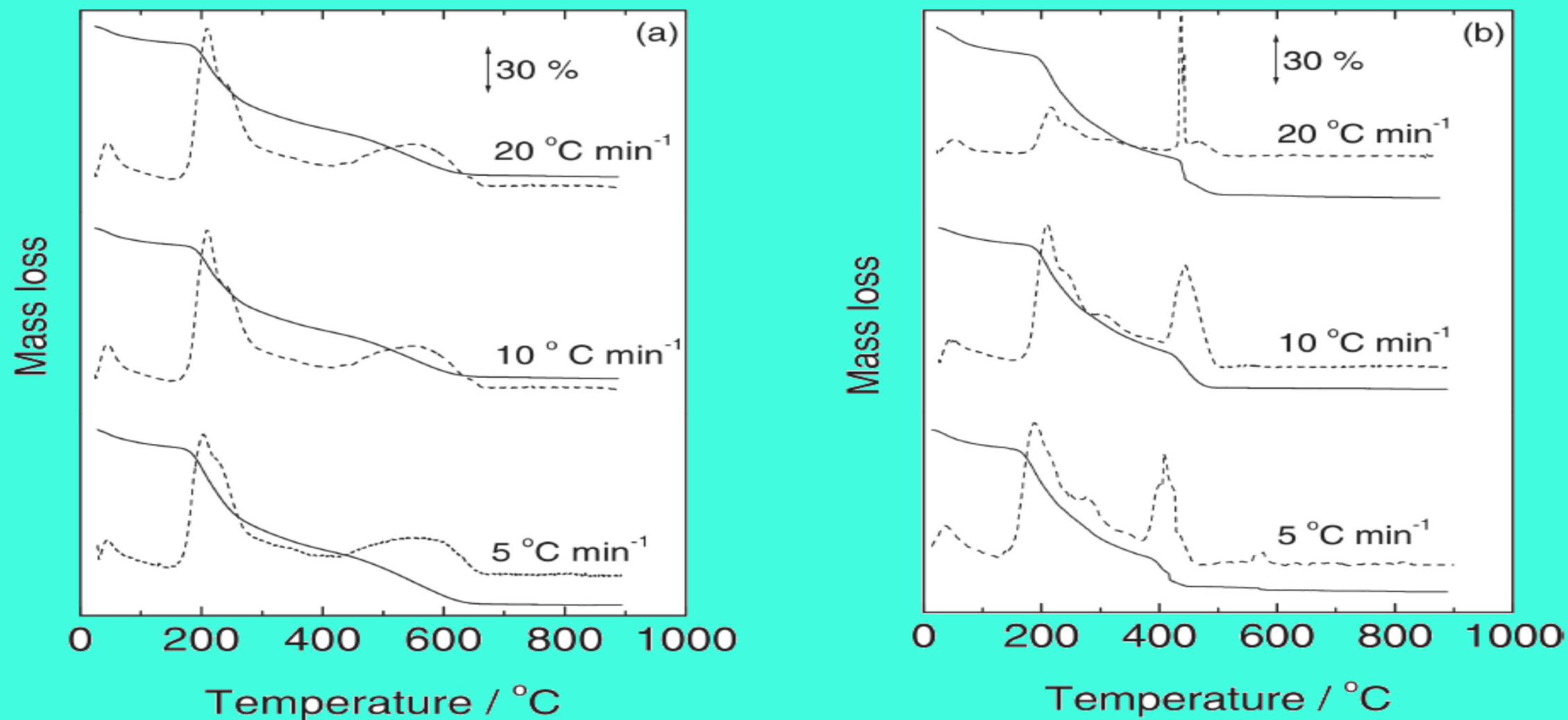


Figure 2. TG (solid)/DTG(dashed) curves of alginic acid in N₂ (a) and (b) under air flow of 90 mL min⁻¹ in different heating rates.

Under N₂ (Figure 4a), HAlg decomposed showing three overlapping peaks. The first endothermic peak suggests the dehydration process at around 80 °C. Then, an endothermic peak close to 200 followed by an exothermic peak at *ca.* 240 °C, respectively, has appeared. Such processes were attributed to the decomposition of the polymer resulting in the carbonized residue, in agreement with TG results.

Figure 4b shows the thermal behavior of the HAlg in air atmosphere. The endothermic peak, centered between 75 and 96 °C depending on the heating rate, may be attributed to the dehydration process. The small endothermic process at 190 °C followed by a strong exothermic peak at *c.a.* 300 °C, correspond to chain decomposition

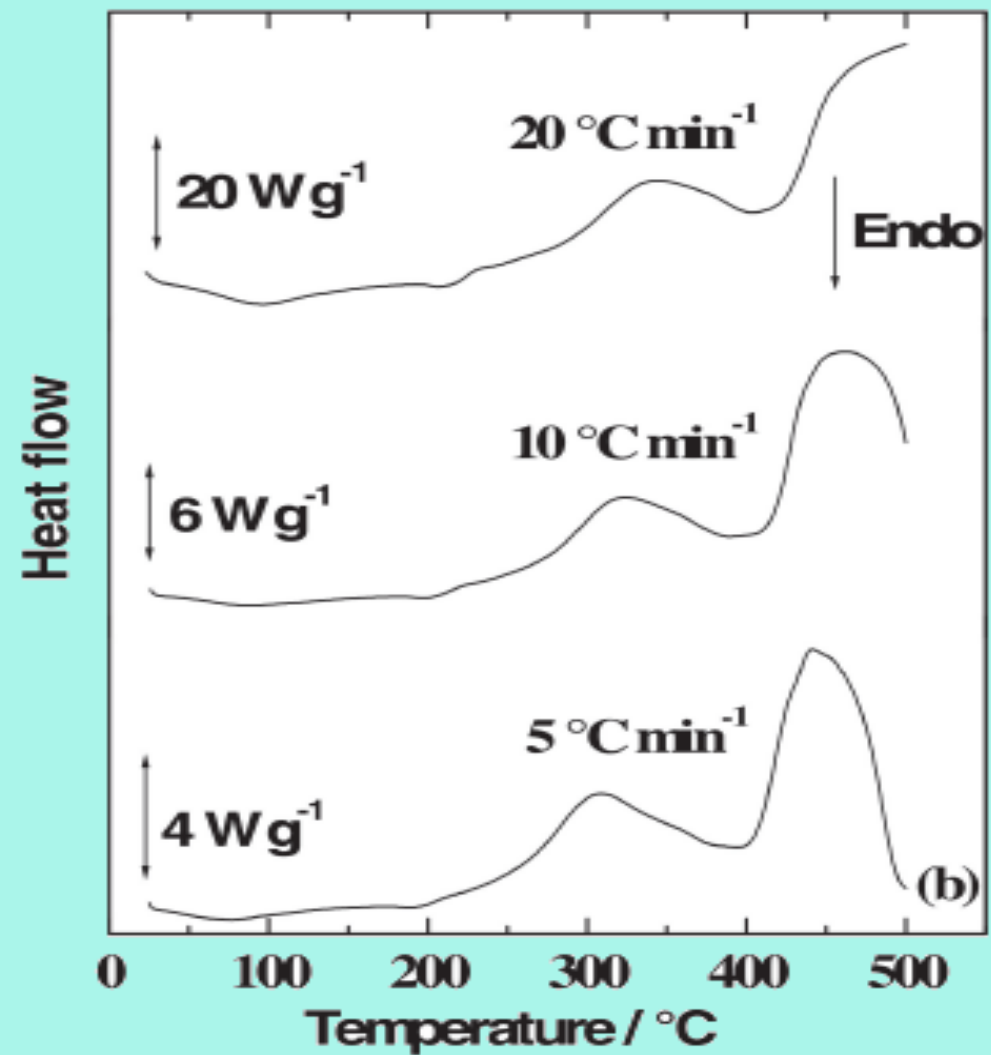
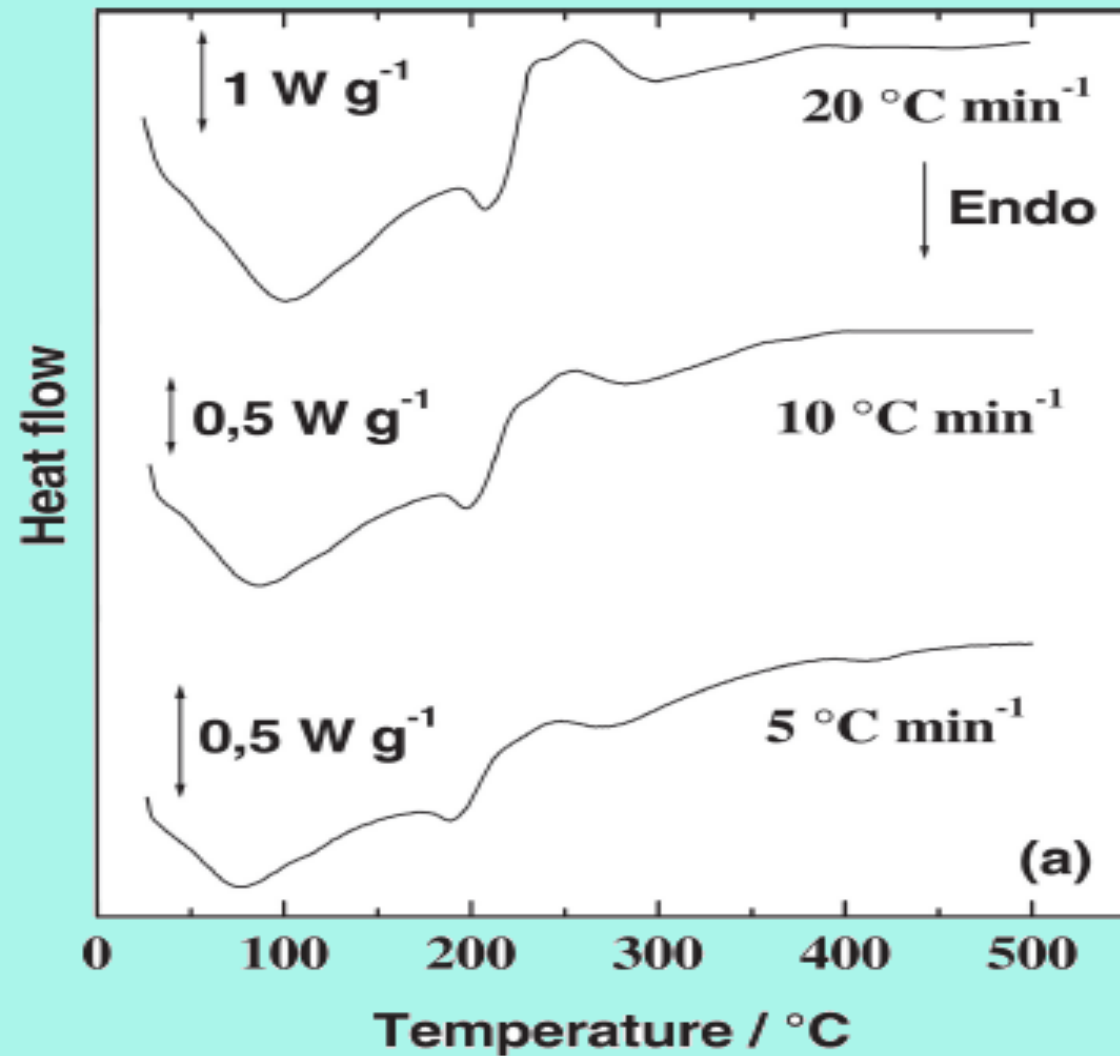


Figure 4 - DSC curves of alginic acid under N_2 (a) and under air (b) flow of $90\ mL\ min^{-1}$ in different heating rates.

The **DSC curves of NaAlg** under N₂ are presented in Figure 5.a. The dehydration was evidenced by an endothermic peak close to 100 °C, then the decomposition of the biopolymer takes place represented by an exothermic peak at c.a. 240-260, depending on the heating rate.

Finally the decomposition of the carbonaceous material occurred above 300 °C.

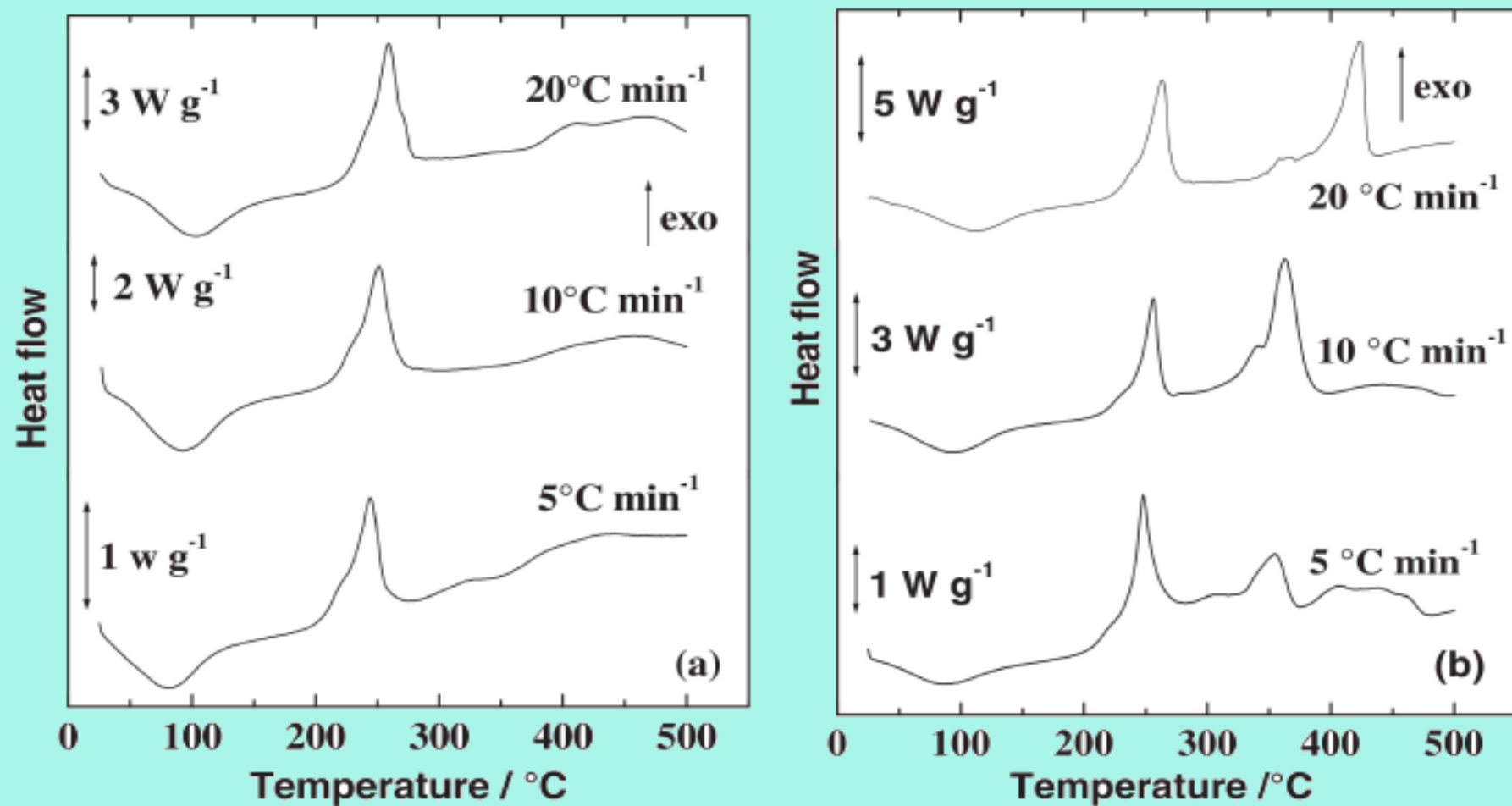


Figure 5. DSC curves the sodium alginate under N₂ (a) and under air (b) flow of 90 mL min⁻¹ in different heating rates.

