

CHAPTER 1

INTRODUCTION

1.1 Background Principle and Rationale

Mungbean (*Vigna radiata* L. Wilczek) has a high level of carbohydrate (62.0-63.0%) and contains a relatively high amount of starch granules (32.0-43.0%). Moreover the infrastructure of mungbean starch (MB) is made up mainly of amylose (32.0-45.0%) (Hoover et.al., 1991; Jung et.al., 1991; Kabir et.al., 1998; Kasemsuwan et.al., 1999; Ohwada et.al., 2003). Amylose is insoluble in water, but becomes gelatinous as the water is heated. Amylose is flexible, transparent, of high strength and homogeneous. Its disadvantage is that it reacts easily toward retrogradation. The result of high amylose levels in starch is instability, though nowadays, chemically-modified starches are used in a broad range of pharmaceutical and medical products as many types of excipient, such as binder, disintegrant, diluent and as the coating of film.

Carboxymethyl starch (CMS), one of the ether derivatives of starch, has been utilized in both of food and non-food industries. Preparation of CMS is carried out through a reaction between MB and monochloroacetic acid (MCA) under alkaline condition. It is completely swelled in cold water, high viscosity transparent and non-toxic. CMS can be prepared from many types of starches, one of them is MB. The literature recommended that carboxymethyl modified mungbean starch (CMMS) had potential as a gelling agent. The CMMS is inert, soluble in cold water, has a suitable viscosity, is stable in 10 freeze-thaw cycles (FT), is good at regulating drug diffusion, non-toxic and safe. CMMS can be used as gelling agent in topical medical gel products (Kittipongpatana et.al., 2006). CMMS gel attains its best properties with high concentration; though the gel is turbid at high concentrations. Another weak point of CMMS is that it is less resistant to alcohol.

The alcohol tolerance of CMMS solution can be improved by cross-linking starch chains so that CMMS is stable in alcohol. The purpose of this study focused on alcohol tolerance ability of cross-linked carboxymethyl modified mungbean starches (CL-MBs) compared with CMMS, cross-linked MB and four commercial polymers (CP, MC, HPMC and SCMC).

1.2 Objectives

1. To improve physico-mechanical properties of carboxymethyl mungbean starch
2. To study the suitable condition for modified starch with carboxymethylation and cross-linking
3. To study the potential use of cross-linked carboxymethyl modified mungbean starch as gelling agent in commercial products
4. To study the physicochemical properties of gel base from cross-linked carboxymethyl modified mungbean starch
5. To develop the gel formulation with active ingredient from cross-linked carboxymethyl modified mungbean starch

1.3 Scope of the study

Native mungbean starch used as the raw material for carboxymethylation and cross-linking was a gift of Sitthinan Co., Ltd., Thailand. The material was in compliance with TIS. 948-2533 standard. The native MB was modified with monochloroacetic acid (MCA) and/or dichloroacetic acid (DCA) in methanol (MeOH). The carboxymethyl group (-O-CH₂-CO-R) was subsequently submitted with MCA via the carboxymethylation reaction and DCA was a cross-linking agent. The modified starches were tested the modified starch identifications, the physicochemical properties, the alcohol tolerability and the stability test. As a result, the three best CL-MBs were chosen to formulate nimesulide (NM) gel. The NM gel formulations were studied the physicochemical properties, the releasing of NM gel formulation and the stability test to show the best formulation that was prepared from CL-MBs.

1.4 Literature reviews

1.4.1 Starch

Starch is a polysaccharide carbohydrate polymer consisting of tens to hundreds to several thousand monosaccharide units. All of the common polysaccharides contain glucose as the monosaccharide unit. Polysaccharides are synthesized by plants, animals, and humans to be stored for food, structural support, or metabolized for energy. Starch is unique among carbohydrates because it occurs naturally as discrete granules (or grains). Starch granules are relatively dense, insoluble and hydrate only slightly in cold water.

Starch is a white, granular, organic chemical that is produced by all green plants. Starch is the large-sized glucose polymer thereby the basic chemical formula of the starch molecule is $(C_6H_{10}O_5)_n$. Anhydroglucose unit is the basis glucose unit that are linked by α -1,4-glycosidic linkage at C#1 of one glucose to C#4 of the next glucose. Anomeric carbon (C#1) is free ending side chain, called reducing end. So, each starch side chain is consisting of 1 position of reducing end (Figure 1.1). Starch is a soft, white, tasteless powder that is insoluble in cold water, alcohol, or other solvents.

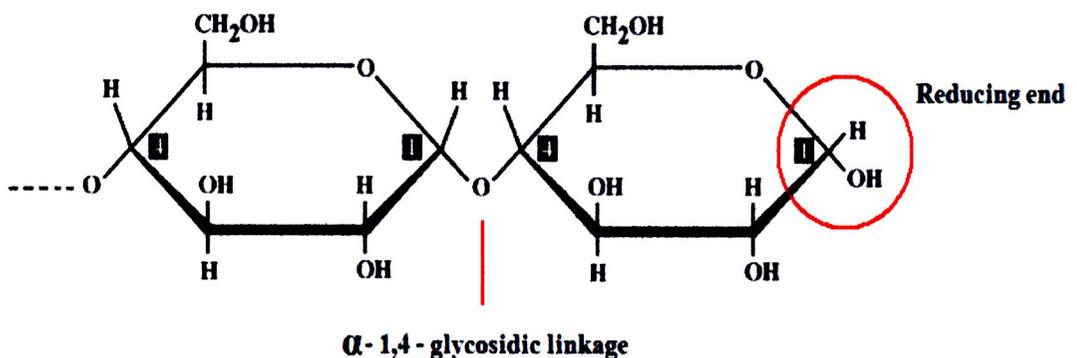


Figure 1.1 The basis glucose unit of starch

The starch granule is mainly composed of a mixture of two polymers: an essential linear polysaccharide, called *amylose* and highly branched polysaccharide called *amylopectin*. Natural starches are mixtures of amylose and amylopectin.

1.4.2 Amylose

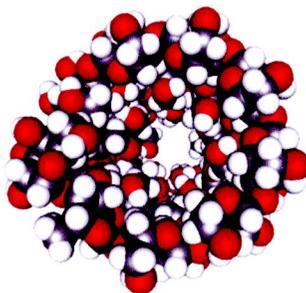


Figure 1.2 Amylose (40-mer of Amylose-All glucose linkages α -1,4)

(Ref.: jchemed.chem.wisc.edu)

Amylose - the constituent of starch in which anhydroglucose units are linked by α -D-1,4-glycosidic linkages to form linear chains (Figure 1.3). Carbon (C) #1 is called the anomeric carbon and is the center of an acetal functional group. A carbon that has two ether oxygens attached is an acetal. The Alpha position is defined as the ether oxygen being on the opposite side of the ring as the C#6. In the chair structure this results in a downward projection. This is the same definition as the -OH in a hemiacetal. The structure of amylose consists of long polymer chains of glucose units connected by an alpha acetal linkage. As a result of the bond angles in the alpha acetal linkage, amylose actually forms a spiral much like a coiled spring. The level of amylose and its molecular weight vary between different starch types. Amylose molecules are typically made from 200-2000 anhydroglucose units. Amylose forms a colloidal dispersion in hot water whereas amylopectin is completely insoluble. Aqueous solutions of amylose are very unstable due to intermolecular attraction and association of neighboring amylose molecules. This leads to viscosity increase, retrogradation and, under specific conditions, precipitation of amylose particles.

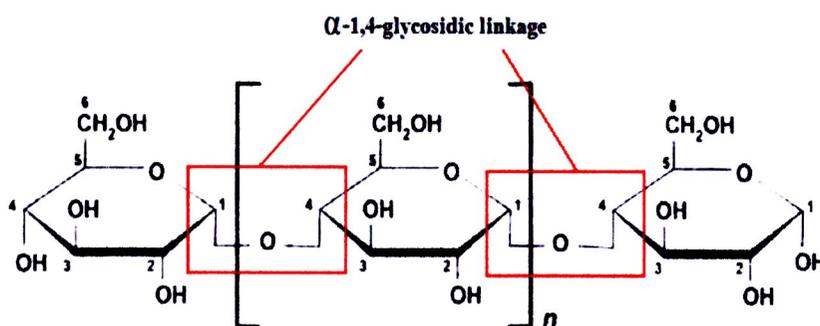


Figure 1.3 The amylose molecule showing the repeating anhydroglucose unit

The average molecular weight of amylose molecule is approximately 106 g/mol and degree of polymerization (DP) of 6000. The chains can easily form single or double helices. The amylose chain gives the molecules a right-handed spiral or helical shape. The inside of the helix contains predominantly hydrogen atom and is lipophilic, while the hydrophilic hydroxyl groups are positioned on the outside of coil (Whistler, 1965). Amylose is responsible for the formation of a deep blue color in the presence of iodine. The iodine molecule slips inside of the amylose coil. Amylose forms a helical complex with iodine giving a characteristic blue color (Table 1.1 and Figure 1.4).

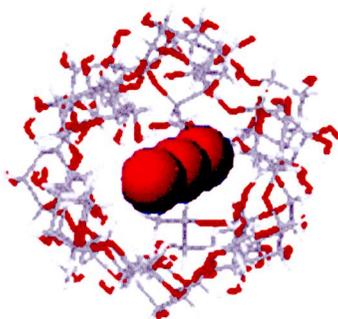


Figure 1.4 Iodine-Amylose Complex

(Ref.: www.chemie.uni-regensburg.de/Organische_Chemie/Didaktik/Keusch/p29_lbm-e.htm
jchemed.chem.wisc.edu)

Table 1.1 The relation of amylose chain length and helical complex with iodine
(Pomeranz, 1985)

Chain length (Glucose unit)	Number of helix urns	Color
12	2	None
12–15 >	2	Brown
20–30	3–5	Red
35–40	6–7	Purple
> 45	9	Blue

1.4.3 Amylopectin

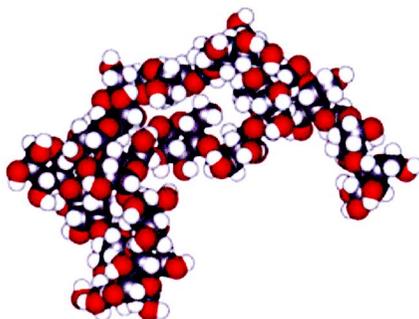


Figure 1.5 Amylopectin fragment with one branch (α -1,6 linkage)

(Ref.: jchemed.chem.wisc.edu)

Amylopectin - the constituent of starch having a polymeric, branched structure. In addition to α -D-1,4- glycosidic linkages which are present in amylose and the linear segments of amylopectin, the amylopectin molecule has α -D-1,6-glycosidic linkages which occur every 20-30 anhydroglucose units (Figure 1.6). The graphic shows very small portion of an amylopectin-type structure showing two branch points. The acetal linkages are alpha connecting C#1 of one glucose to C#4 of the next glucose. The branches are formed by linking C#1 to a C#6 through an acetal linkages. Amylopectin has 12-20 glucose units between the branches. Natural starches are mixtures of amylose and amylopectin. Aqueous solutions of amylopectin are characterized by high viscosity, clarity, stability, and resistance to gelling.

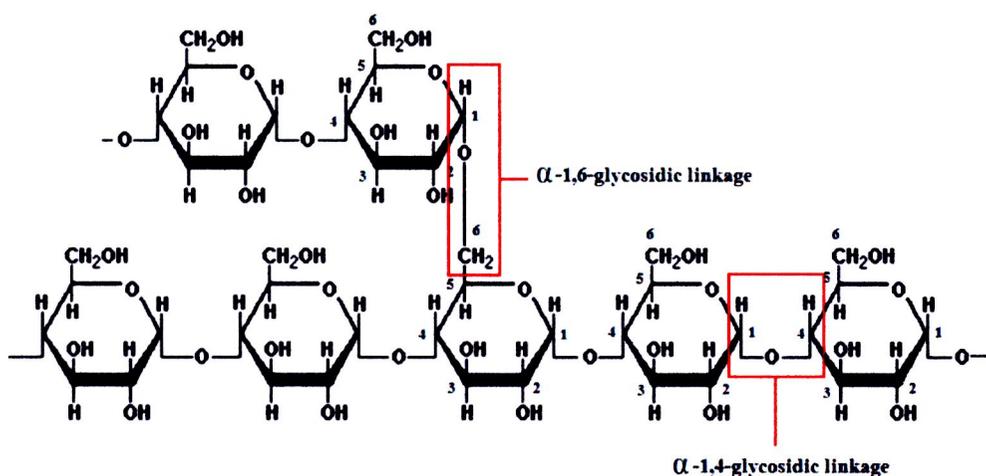


Figure 1.6 The amylopectin molecule showing the two different types of chain linkages

Amylopectin binds weakly with iodine and the complex typically gives a red/brown color. The level of amylopectin varies between different starch types. Waxy starches are almost 100% amylopectin. As a result, the iodine complex is specific to detect the amylose. The amylose content of starch was determined by the colorimetric method.

Properties of these two major starch components are summarized in Table 1.2.

Table 1.2 Properties of amylose and amylopectin components of starch
(Beynum et. al, 1985)

Properties	Amylose	Amylopectin
General structure	Essentially linear	Branched
Glycosidic linkages	α -1,4	α -1,4 and α -1,6
Average chain length (glucose residues)	100–10000	20-30
Degree of polymerization (glucose residues)	200-2000	> 10000
Solubility in water	Variable	Soluble
Solubility in aqueous solution	Retrogrades	Stable
λ_{\max} of iodine complex	~ 650 nm	~ 540 nm
Iodine affinity	19–20 %	< 1 %
Color with iodine	Dark blue	Purple
Character after heating	Gel – liked appearance	No gel

1.4.4 Starch gelatinization

Starch gelatinization is a process that breaks down the intermolecular bonds of starch molecules in the presence of water and heat, allowing the hydrogen bonding sites (the hydroxyl hydrogen and oxygen) to engage more water. This irreversibly dissolves the starch granule. Penetration of water increases randomness in the general granule structure and decreases the number and size of crystalline regions. Crystalline regions do not allow water entry. Heat causes such regions to be

diffused, so that the chains begin to separate into an amorphous form. Under the microscope in polarized light starch lose its birefringence, its extinction cross. The process of gelatinization can summarize:-

- Gelatinization is also known as the thickening of a liquid.
- The starch grains or flour granules absorb the liquid.
- When heated the grains/granules swelling and then burst, releasing starch into the liquid.
- The granules or grains swell to 30 times their original size (swelling power, peak viscosity).
- Prolonged heating and/or pressure and stirring is needed to completely dissolve the rest fragments of starch granules. The viscosity will reduce and rheology-texture of the solution will change.

The gelatinization temperature of starch is depending on plant type, amount of water, pH, types and concentration of salt, sugar, fat and protein in the recipe, derivertization technology used. Some type of unmodified native starches start swelling at 55°C, other types at 85°C. Gelatinization temperature depends also on the amount of damaged starch granules. These will swell faster. Damaged starch can be produced for example during the wheat milling process or when drying the starch cake in the starch plant.

1.4.5 Starch retrogradation

Cooked unmodified starch when cooled for long enough period, will thicken or gel and rearrange themselves again to a more crystalline structure, this process is called retrogradation. Retrogradation is a reaction that takes place in gelatinized starch when the amylose and amylopectin chains realign themselves, causing the liquid to gel. When native starch is heated and dissolves in water, the crystalline structure of amylose and amylopectin molecules are lost and they hydrate to form a viscous solution. If the viscous solution is cooled or left at lower temperature for long enough period, the linear molecules, amylose, and linear parts of amylopectin molecules retrograde and rearrange themselves again to a more crystalline structure. The linear chains place themselves parallel and form hydrogen bridges. In viscous solutions the viscosity increases to form a gel. At temperatures between -8 and +8°C the aging process is enhanced drastically. Retrogradation can

expel water from the polymer network. This is a process known as syneresis. A small amount of water can be seen on top of the gel.

Chemical modification of starches can reduce/enhance the retrogradation. Waxy, high amylopectin, starches have also much lesser tendency to retrograde. Also additive as fat, glucose, sodium nitrate and emulsifier can reduce retrogradation of starch.

1.4.6 Starch modification

Native starch is insoluble in water and has specific characteristics that make it inappropriate to use in the industry. Hence, starch modification is the first choice used to improve the starch properties for using in the industry.

Modified starch is defined as “the product of starch (such as corn starch, tapioca starch, wheat starch or potato starch etc.) was employed to change or alter the physical and/or chemical properties of native starch by heating and/or enzyme and/or chemicals for the useful benefits in each industry”. The definition is given by Thai Industrial Standards Institute with TIS 1037-2535.

Modified starch or starch derivatives, are prepared by enzymatically, physically, or chemically treating native starch, thereby changing the properties of the starch. Modified starches are used in practically all starch applications, such as in food products as a thickening agent, stabilizer or emulsifier; in pharmaceuticals as a disintegrant; or in paper as a binder. They are also used in many other applications. Starches are modified to enhance their performance in different applications. Starches may be modified to increase their stability against excessive heat, acid, shear, time, cooling, or freezing; to change their texture; to decrease or increase their viscosity; to lengthen or shorten gelatinization time; or to increase their visco-stability.

Starch modification can be categorized into 3 types (Whistler, 1965) that comprise of chemical modification, physical modification and biological modification.

1. Chemical modification

- 1) Derivatization : starch is substituted by esterification reaction (as starch acetate), by etherification (as hydroxyalkyl starch and carboxymethyl starch), by cross-linking (as cross-linked starch)
- 2) Acid thinning : e.g. acid-modified starch
- 3) Dextrinization : e.g. dextrin

- 4) Oxidation : e.g. oxidized starch
 - 5) Hydrolysis : e.g. maltodextrin
2. Physical modification:-
- 1) Gelatinization : e.g. pregelatinized starch
 - 2) Granular-Cold-Water-Soluble-Starch (GCWSS)
 - 3) Mechanical resized starch
 - 4) Annealing
 - 5) Heat moisture treatment
3. Biotechnological modification:-
- 1) Low-amylose starch, called waxy starch
 - 2) High-amylose starch

The chemical starch modification is the most common technique employed to change or alter the starch properties. This modification can specify the modified starch properties for useful benefits in each industry which are:

1. The gelatinization temperature of modified starch is lower than native starch.
2. Modified starch can be absorbed, swelled and soluble more than native starch.
3. The viscosity of modified starch is depended on substitution into the starch molecule.
4. Modified starch is a stabilizing agent because the chemical modification can reduce the retrogradation of starch and be stable at freeze-thaw stability test.

1.4.7 Carboxymethylation

Starches can have a hydrogen atom replaced by something else, such as a carboxymethyl group, making *carboxymethyl starch* (CMS) (Figure 1.7). CMS is prepared by carboxymethylation reaction between native starch and MCA under the alkaline condition (Figure 1.8). Adding the carboxymethyl group makes the starch less prone to damage by heat and bacteria. CMS is used as an additive in oil drilling mud, and is used in the goo that makes ultrasound examinations messy. CMS is also called a starch ether.

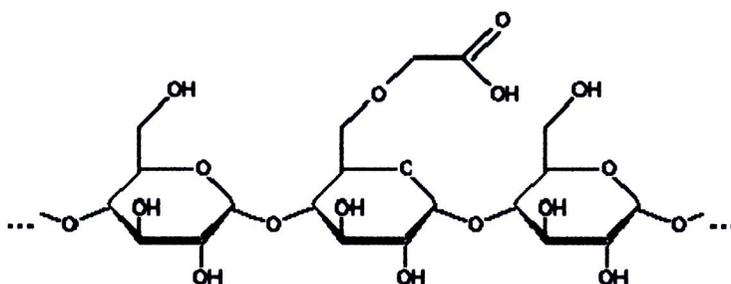


Figure 1.7 Carboxymethyl starch (CMS)

Carboxymethyl groups make the starch more hydrophilic, and aid in cross-linking. This makes CMS useful in other tablets to make them disintegrate quickly. Longer carbon side chains can also be added, such as carboxyethyl groups, or carboxypropyl groups. Adding bulky functional groups like carboxymethyl and carboxyethyl groups reduces the tendency of the starch to recrystallize. When the starch stays as a gel, a product is softer. When the starch regains its crystalline form, the product becomes firmer. The technical term for this phenomenon is starch retrogradation. CMS is completely swelled in cool water, high viscosity transparent and non-toxic, water-soluble and forms gel with decent clarity and strength. CMS can be used widely such as binder or disintegrant in pharmaceutical industry, glue, paper coating etc.

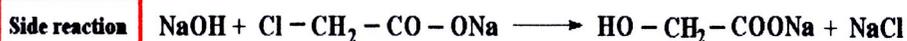


Figure 1.8 Carboxymethylation reaction

The literature review by Kittipongpatana et.al. (2006) recommended that carboxymethyl modified mungbean starch (CMMS) had potential as a gelling agent. CMMS was prepared by etherification process with carboxymethylation reaction that was the substitution of carboxymethyl group into native MB molecule with MCA. The process was reacted by using MeOH as a solvent or medium under the alkaline condition at 70°C for 60 min. The CMMS is inert, soluble in cold water, has a suitable viscosity, is stable in 10 freeze-thaw cycles, is good at regulating drug

diffusion, non-toxic and safe. CMMS can be used as gelling agent in topical medical gel products.

In the study by Kittipongpatana et.al. (2009), CMMS was used as a gelling agent for the topical non-steroidal anti-inflammatory drugs (NSAIDs). The appearance of the formulation was clear, homogenous, non separation or precipitation, no greasy and could be washed out. The formulation was stable under the accelerated condition. The rheogram of gel exhibited a pseudoplastic with thixotropy. The formulation would be shown the perfect releasing profile similar with the commercial polymer at the high concentration. Therefore, CMMS gel attains its best properties with high concentration; though the gel is turbid at high concentrations. Another weak point of CMMS is that it is less resistant to alcohol. The alcohol tolerance of CMMS solution can be improved by cross-linking starch chains so that CMMS is stable in alcohol. The process was called *cross-linking*.

1.4.8 Cross-linking

Cross-linking is the reaction between native starch and a cross-linking agent at 45-50°C that lower than the gelatinization temperature. The reaction just occur at the surface of starch granule. When the starch granule is cross-linked, the internal grain structure will strengthen that a cause of increasing the gelatinization temperature for the starch granule swelling. The areas with a crystal structure mainly determine the solidity of grains and thus the gelatinization temperature. The modified starch from the reaction is called cross-linked starch.

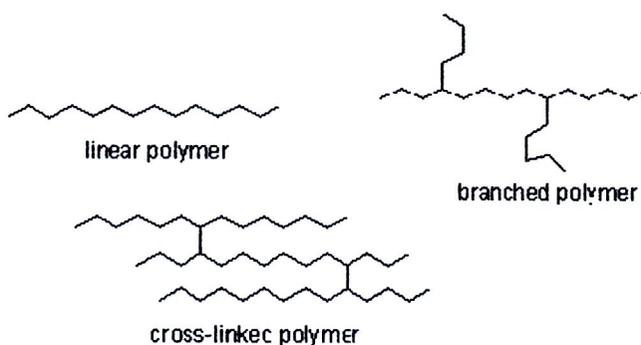


Figure 1.9 Cross-linked starch

(Ref: www.sep.alquds.edu/chemistry/scripts/Student/Exp_1_files/image006.gif)

The cross-linking can occur between molecules in a solution or in the starch granule when two polymers (due to a reaction) are compounded. The effect of cross-linking is considerable. This can easily be imagined by keeping in mind that 1 cross-linked can signify the doubling of the molecule weight. The main effects which cause cross-linking are:-

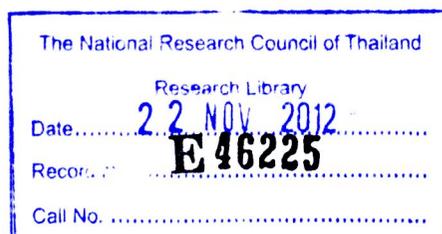
1. Increasing the gelatinization temperature.
2. Changing the viscosity.
3. Changing the character of starch solutions.
4. Reducing the sensitivity to stirring forces.



By cross-linking a second kind of solidity is brought into the structure, which we can adjust to any level. It is even possible to take cross-linking that far that the granule will not swell again under any condition. With a less far cross-linked starch the granule swells to a maximum value while heated. Through cross-linking the granule remains an entity for a longer period. This increases the viscosity maximum and the temperature at which the maximum is found shifts to a higher value.

Cross-linking occurs when a hydroxyl group (-OH) on one chain bonds with a hydroxyl group on an adjacent chain. This toughens the starch, and helps it resist heat and acids but reduce the starch swelling. As a result, cross-linking is cooperated with other chemical modification in order to prepare the useful benefits modified starch to support.

Cross-linking can be done by reacting with compounds such as sodium trimetaphosphate, epichlorhydrin, glutaraldehyde, and phosphorous oxychloride, the new one with the less study is *dichloroacetic acid (DCA)*. The sample reaction of cross-linking is shown in Figure 1.10.



of ethanol (EtOH)], alkalized with 150 mL of 45% sodium hydroxide (NaOH) at 20°C for 30 min and continuously stirring. Both, carboxymethylation and cross-linking were then carried out simultaneously, using the *Williamson* etherification reaction. This was done by adding MCA for carboxymethylation and DCA for cross-linking, and stirring the mixture at 50°C for 3 h. After neutralizing with 5 N aqueous hydrochloric acid (HCl) and washing out the generated sodium chloride (NaCl) with 80% alcoholic solution. The product was dried in an oven at 60°C and ground (using a 500 mm mesh). Cross-linking CMPS was resist to shear stress and any stimulant, less set-back phenomenon and high liquid absorption. So, it was a potential super-absorbers and can be used widely in the medical and pharmaceutical industry. Moreover, cross-linked CMPS that prepared from PS was non-toxic and irritate to human skin.

Christoph et.al (2007), cross-linked CMPS varied in their degree of substitution (DS), as average number of carboxymethyl groups per monomer unit. As a result, after neutralizing and removal of the formed salt, one part of the synthesized CMPS networks was pre-swollen in water in an additional purification step in order to wash out unlinked, soluble polymer chains. Different swelling experiments were performed with the two samples (washed samples and unwashed samples), before being dried and ground. The Free Swelling Capacity (FSC) of the hydrogels were strongly influenced by chemically unlinked CMPS chains that was only physically complicated perplex in the network structure. These mobile polymer segments were responsible for a significant weight loss of the swollen, unwashed hydrogels over the course of time. Rheological experiments showed that the polymer content of an unwashed hydrogel had to be more than twice as high as that of the corresponding purified product. CMPS gels from washed samples were stable more than unwashed samples after the cyclic temperature program and long-term stability test. Hence, washed samples or the purified CMPS gels could be used as a gelling agent in medical ultrasonic diagnostics, as they are toxicologically unobjectionable for human beings.

1.4.9 Nimesulide

Nimesulide (NM) is a NSAIDs characterized by the presence of a sulfonanilide moiety as the acidic group rather than a carboxylic group or chemically

4'-nitro-2'-phenoxy methane sulfonanilide (Figure 1.11), is a weakly acidic non-steroidal anti-inflammatory drug. It differs from other NSAIDs, NM shows high anti-inflammatory, antipyretic and analgesic activities in addition to low toxicity, a moderate incidence of gastric side effects, and a high therapeutic index. NM is a relatively weak inhibitor of prostaglandin synthesis in vitro and appears to exert its effect through a variety of mechanisms that show in Table 1.3. It also exhibits a significant selectivity toward cyclooxygenase-2 (COX-2) versus COX-1 inhibition, which selectively inhibit COX-2 are able to be of symptomatic benefit without affecting the gastric mucosa. However, recent findings reported that NM has a higher risk of hepatotoxicity when compared to other marketed NSAIDs. NM has been withdrawn from market in many countries. Hence, the NM gel topical NSAIDs is the choice to abstain from a hepatotoxicity.

Table 1.3 Mechanisms of action of nimesulide (Zuanetti, 2000).

-
- Inhibits prostaglandin synthesis selectively (preferentially inhibiting COX-2).
 - Interferes with the production and/or action of several enzymes (e.g. elastase, collagenase), derivatives (free radicals), cytokines, toxic oxygen, histamine and platelet activating factor (PAF).
 - Increases intracellular cyclic adenosine monophosphate (cAMP), thus inhibiting PAF synthesis and release of from the neutrophils.
 - Inhibits the synthesis of cartilage degrading enzymes (collagenase, stromelysin and metalloproteases).
 - Inhibits the synthesis of interleukin-6 and urokinase.
 - Induces intracellular phosphorylation and activation of glucocorticoid receptors and activates their binding to target leukotriene B genes.
-

In addition to its anti-inflammatory properties, it possesses both analgesic and antipyretic properties. As other members of this drug class, the major effects of nimesulide occur through the inhibition of synthesis of prostanoids (prostaglandins and tromboxanes) from arachidonic acid by the enzyme cyclooxygenase (COX). It has been recently discovered that COX has two isoforms, the constitutive COX-1 and

the inducible COX-2. The former is normally active in all tissues, but it is of particular importance in the stomach, kidney and platelets, where the prostanoids produced by COX-1 are protective. For example, in the stomach they contribute to maintain gastric mucosa integrity by various processes such as maintenance of tissue blood flow. In contrast, COX-2 is mainly present in the leukocytes and is induced by inflammatory stimuli (some of the cytokines, mitogens and endotoxins). Thus, it is responsible for the synthesis of the prostanoids involved in pain and inflammation in inflamed tissues. NSAIDs are thought to damage the gastric mucosa mainly by inhibiting prostaglandin synthesis in the stomach, whereas their inhibition at inflamed sites relieves pain and inflammation. Collectively, the available data indicate that increasing the COX-2 selectivity of NSAIDs gives a very favorable gastrointestinal safety profile and offers a better prospect in the treatment of rheumatic conditions and musculoskeletal disorders, combining efficacy with safety.

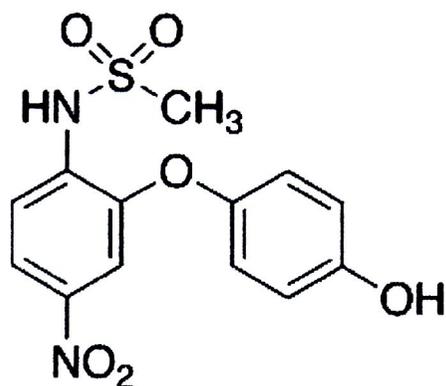


Figure 1.11 Chemical structure of nimesulide