Chapter I

General Background

Introduction

Microencapsulation can be described by Bakan,1994 as a process in which very thin coating of polymeric material(s) is deposited around particles of solids or droplets of liquids. The microcapsules thus formed range dimensionally from several nanometers to several thousand nanometers.

The microcapsules have been prepared by a variety of methods. The methods of preparation and the techniques were catergorized by Li et al. (1988) as follows : phase separation or coacervation, interfacial polymerization, electrostatic methods, and mechanical methods.

Coacervation is a common method of microencapsulation (Burgenberg de Jong, 1949 ; Nixon & Nough, 1978 ; Burgess & Carless, 1985). Microencapsulation by coacervation-phase separation can use non aqueous or aqueous vehicles which are safe and less toxic. It was first developed commercially by the National Cash Register Co. (NCR), in 1954. The coacervation has been classified into two categories : simple and complex coacervations. Simple coacervation concerns with only one colloid. The process involves the addition of a strongly hydrophilic substance to reduce the solubility of the macromolecule and cause two phases to be formed. Microencapsulation by complex coacervation involves the use of more than one colloid and concerns with the charges. The wall

is formed by electrostatic interaction between the positive charge of the polymer chain and the negative charge on the counter polyanion backbone.

Microencapsulation for peroral controlled release dosage form which uses hydrophilic colloid as wall material has advantages over the method involving hydrophobic colloid in the way of using less or non toxic solvents. So, this research would emphasize the complex coacervation process which involves the use of aqueous solvent and ionic water soluble polymers as the coating materials. This process is based on a cationic polymer complexing with an anionic polymer, thus forming a coating polymer complex. It is designed to microencapsulate a variety of water-insoluble liquids, solids, solutions and dispersions. Recently, a hydrophilic cationic polyelectrolyte polymer has shown to be successfully used as wall material for controlled release microspheres and microcapsules (Thanoo, Sunny, and Jayakrishnan, 1992 ; Polk et al., 1994). Chitosan (Skaugrud, 1991 ; Skaugrud, 1989 ; Lower, 1984) is a biocompatible and non toxic biopolymer, which hence lends itself to be beneficially used in pharmaceutical applications. Counter polyanions used in this study were carboxymethylcellulose or pectin to form complex coacervation with chitosan and the characteristics of their wall materials were compared. The model drug was indomethacin, water-insoluble drug.

Chitosan-carboxymethylcellulose microcapsules prepared by complex coacervation technique for controlled release dosage forms was studied by Ritthidej and Tiyaboonchai (1995). The study determined the effects of processing variables such as pH, hardening time and amount of glutaraldehyde on the physical properties and drug release pattern. The report revealed that the optimum temperature to form microcapsule wall was less than 15°c. The other optimum variables of preparation conforming the USP drug release specification were pH 3.0 and 4.0, 3 hours for

hardening time and 0.25 gm glutaraldehyde content /gm polymer. Moreover, microencapsulation of indomethacin by complex coacervation of chitosan-CMC could elucidate the effect of chitosan or CMC concentration on the physical properties of microcapsules. However, no studies have been published on using chitosan-pectin microcapsules prepared by complex coacervation technique for controlled release dosage form. This study determined the effect of chitosan and pectin concentration, hardening time and glutaraldehyde content on the physical properties and drug release pattern and compared the physical properties and the release pattern with the chitosan-CMC indomethacin microcapsules which controlled drug release of 24 hours.

Objectives of the study

1. To study the optimum processing conditions for the preparation of indomethacin microcapsules by complex coacervation of chitosan-carboxymethylcellulose and chitosan-pectin.

2. To determine and compare the physicochemical characteristics of indomethacin microcapsules preparing from chitosan-carboxymethylcellulose and chitosan-pectin.

3. To obtain the optimum formulation of indomethacin controlled release microcapsules.

4. To study the reproducibility of physical properties and drug release pattern in consecutive batches.

Literature Review

Microencapsulation :

Microencapsulation is a means of applying relatively thin coatings to small particles of solids or droplets of liquids and dispersions. Microencapsulation processes (Nixon, 1980 ; Li et al., 1988 ; Bakan, 1994) that are most applicable to pharmaceuticals are given in Table 1, which cites the process, the core materials which can be coated, and the approximate size range of the microcapsules that can be manufactured.

Table 1 : Microencapsulation processes and their applicability

Process	Core material	Microcapsule size (nm)
Air suspension	solids	35-5000
Coacervation-phase separation	liquids and solids	1-5000
Multiorifice-centrifugal	liquids and solids	1-5000
Pan coating	solids	600-5000
Solvent evaporation	liquids and solids	1-5000
Spray drying and congealing	liquids and solids	5-600

Properties of microcapsules : (Bakan, 1994)

Microcapsules can be single-particle or aggregate structures. They can vary in size from 1 to 5000 nm. A unique feature of microcapsules is the smallness of the coated particles and their use and adaptation to a wide variety of dosage

forms. Because of the smallness of the particles, drugs can be widely distributed throughout the gastrointestinal tract, thus improving drug absorption.

The core material plays a significant role in the production of microcapsules. It dictates the process as well as the polymer used as the coating material. It should be insoluble and nonreactive with the coating material and the solvent. The microcapsule coating can be chosen from a wide variety of natural and synthetic polymers. Physical characteristics of the polymer coating can be varied to some extent, that is, the coating can be made rigid, fragile or strong. Strength is controlled by the choice of polymer, coating thickness and coating plastization.

Applications : (Madan, 1978 ; Kondo, 1979 ; Deasy, 1984 ; Li et al., 1988)

Microencapsulation has been applied to develop pharmaceutical dosage forms. Bakan (1994) has summarized some applications including converting liquids to solids such as flavors, fish oils, vegetable and silicone oils, as well as vitamins ; separation of incompatible components in a dosage form, for example, the reduction of salicylic acid when microencapsulated aspirin and propoxyphene hydrochloride are admixed ; taste-masking of bitter drugs by film coats the drug particle to prevent contact with the taste sensors upon ingestion ; controlled-release medications such as aspirin, theophylline ; reduction of gastric side effects of some drugs such as potassium chloride, aspirin.

Markel (1984) reported new applications for parenteral sustained release. These applications were the inclusion of proteins, hormones, enzymes, antigens, cytotoxic drugs, radiolabelled drugs, and vaccines into microcapsules.

Phase separation-coacervation :

Encapsulation by coacervation was an uncomplicated process which did not involve any elaborate manufacturing equipment. This process was developed in the early 1950 s by Barrett K. Greess (Green, 1957) at what was then known as the National Cash Register Co. (NCR). This first successful commercial development of a product using microcapsules was carbonless copy paper which eliminated the need for the carbon paper in multipart business forms. The first pharmaceutical product using microcapsules was a controlled-release aspirin product. (Bell et al., 1966)

The formation of microcapsules by the method of coacervation had three steps as follows : (Luzzi, 1970 ; Bakan, 1994)

1. Formation of three immiscible phases, the liquid-vehicle phase, the core material, and the liquid polymer coating;
2. Deposition of the coating material;
3. Solidification of the coating material.

The coating material was formed by utilizing one of the methods of phase separation or coacervation by simple or complex coacervation. Simple coacervation was induced by a change in conditions which resulted in molecular dehydration of the macromolecules. This may be achieved by the addition of microions, the addition of a soluble salt such as sodium or ammonium sulfate, or a temperature change, all of which promoted polymer-polymer interactions over polymer-solvent interactions. Complex coacervation was driven by electrostatic interactive forces between two or more macromolecules. The deposition of the coating material was

promoted by a reduction of the total free interfacial energy of the system, brought about by a decrement of the coating material surface area during coalescence of the liquid polymer droplets. The coating could be hardened in a variety of ways be thermal, crosslinking, or desolvation methods, to form a rigid microcapsule.

Complex coacervation :

Complex coacervation processes involved the use of water as the solvent phase and ionic water-soluble polymers as the coating material. This process was based on electrostatic interactions between the cationic polymer and the anionic polymer to form complex.

Burgenberg (1949) concluded that a large number of variables involved in complex coacervation such as pH, ionic strength, macromolecule concentration, macromolecule ratio, and molcular weight affected microcapsule production, resulting in a large number of controllable parameters. Complex coacervate systems that were investigated as potential microencapsulation methods included gelatin-acacia (Kondo, 1979 ; Nixon and Hasson, 1980), gelatin-carbopol (Elgindy and Elegakey,1981 ; Elegakey and Elgindy, 1983), gelatin-pectin (McMullen, Newton, and Becker, 1982 ; McMullen et al., 1984), gelatin-gelatin (Burgess and Carless, 1985), gelatin-carboxymethylcellulose (Koh and Tucker, 1988), albumin-acacia (Burgess, Kwok, and Megremis, 1991 ; Burgess and Singh, 1993), chitosan-alginate (Polk et al., 1994) and chitosan-carboxymethylcellulose (Ritthidej and Tiyaboonchai, 1995). Complex coacervate microcapsules could be formulated as suspensions or gels (Calanchi, 1976) and could be compounded within suppositories (Umeda et al., 1983 ; Nakajima et al., 1987) and tablets (Nakajima et al., 1987).

Formulation variables and processing condition variables :

Several papers have been published describing the effects of formulation variables and processing condition variables on physicochemical properties and drug release patterns of microcapsules. Formulation and processing condition variables are core material, wall material or polymer such as concentration and molecular weight, viscosity, crosslinking agent, hardening time, pH, temperature, ionic strength, stirring rate, feed rate, etc. These variables are described as follow :

Core material : Core materials such as gaseous, liquid and solid may be contained in microcapsules, and the material to be contained may be either hydrophilic or hydrophobic. For complex coacervation using aqueous vehicle, the core material is a water-insoluble and inert to the wall material. Milovanovic and Nairn (1986) concluded the result of microencapsulation of sulfadiazine by cellulose acetate phthalate that when the amount of drug increased, the diameter of the microcapsules also tended to increase. Akbuga and Durmaz (1994) prepared chitosan furosemide microspheres from W/O emulsion systems and the result showed that as the amount of drug incorporated increased, furosmide release also increased.

Wall material : A variety of inorganic and organic materials can be used as wall materials, but polymeric substances are used most frequently. The wall material is selected appropriately depending upon the physical properties of the core material. If the core material is oleophilic, a hydrophilic polymer is used as the wall materials. When an aqueous solution is used as the core material, a water-insoluble synthetic polymer is used as the wall material. Optimum concentration ratio of polymer to form interpolymer complex are confirmed by minimum

viscosity, maximum turbidity, maximum coacervate volume, and electrophoresis. The systems investigated by these measurements were gelatin-acacia by Bungenberg de Jong (1949), carbopol-gelatin by Elgindy and Elegakey (1981), pectin-gelatin by McMullen et al. (1982), gelatin-gelatin by Burgess and Carless (1985), carboxymethylcellulose-gelatin by Koh and Tucker (1988), hydroxypropylcellulose-carboxyvinyl polymers by Satoh et al. (1989), chitosan-pectin and chitosan-acacia by Meshali and Gabr (1993), chitosan-sodium alginate and chitosan-sodium polyacrylate by Takahashi et al. (1990), and chitosan-sodium hyaluronate by Takayama et al. (1990).

Ritthidej and Tiyaboonchai (1995) concluded that the concentration of chitosan solution had no effect on the drug release from the chitosan-carboxymethylcellulose microcapsules. Polk et al. (1994) found in chitosan-alginate microcapsules that chitosan concentration had an effect on expansion factor in contrary to chitosan molecular weight which had no effect. Increasing the molecular wight of chitosan and the alginate concentration would be able to reduce the elution of albumin out of the capsule. McKnight et al. (1988) concluded that the molecular weight of chitosan employed in the encapsulation procedure was determined to be a key factor in the capsule strength and flexibility. Goosen et al. (1989) reported that membranes formed with reduced molecular weight chitosan were strong and flexible.

Crosslinking agent : Hou et al. (1985) stated that aldehyde is known as a reagent for crosslinking and schiff's base formation. Glutaraldehyde and formaldehyde are commonly used as crosslinking agents for protein-polysaccharide complex coacervate systems such as gelatin and acacia (Baker, 1994). A condensation reaction occurs between the amino groups of the protein and the

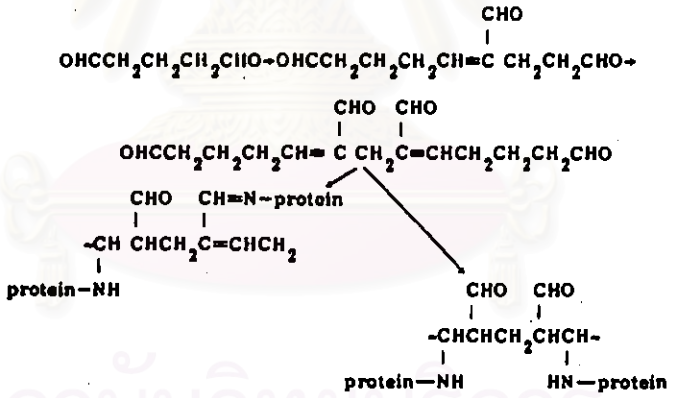
aldehydes. The drug release from microcapsules generally decreased with the increment in glutaraldehyde content until a point was reached where a sharp drop to the minimum release rate occured then the rate increased again with the increasing content. The initial dropped in drug release when increasing the glutaraldehyde content was because the increased amount of glutaraldehyde helped strengthen and increased the tortuosity of the microcapsule wall membrane in the crosslinking process. Luzzi and Gerraughty (1967) observed that excesssive hardening treatment increased drug release, possibly by excessively denaturing and cracking of wall. The results were in agreement with other investigators. (Nakatsuka and Andrady, 1992 ; Ritthidej and Tiyaboonchai, 1995)

Nixon and Hassan (1980) found that hardening with formaldehyde produced slightly larger microcapsules and wider size distribution. The formaldehyde effect was probably due to the crosslinking of the gelatin producing a more rigid wall which did not reduce in thickness during the dehydration stage of preparation. Takenaka, Kawashima, and Lin (1979) investigated the effect of amount of formaldehyde as hardening agent on the in vitro release of sulfamethoxazole from microcapsules prepared by gelatin-acacia complex coacervation. They showed that increasing formalin treatment tended to decrease the dissolution rate of the drug from the microcapsules. Thacharodi and Rao (1993) investigated the chitosan membrane between crosslinked and uncrosslinked membrane. They found that the surface of the crosslinked chitosan membrane appeared very smooth and compact whereas the uncrosslinked membrane showed a porous and granular structure. Moreover, IR spectrum of uncrosslinked membrane showed a strong peak of the primary amino group at 1574 cm^{-1} whereas, in the crosslinked chitosan membrane, a doublet between 1660 and 1590 cm^{-1} appeared,

which is characteristic of the C=N group. The IR spectra clearly indicated the process of a schiff's base type of crosslink in the glutaraldehyde-treated membrane.

Morse (1971) in a patent assigned to Merck and Co., Inc., microencapsulated indomethacin by complex coacervation using glutaraldehyde as the crosslinking agent to obtain microcapsules that had a sustained release effect in simulated gastric fluid.

Glutaraldehyde is considered a better crosslinking agent for protein than formaldehyde. Aqueous solutions of glutaraldehyde are largely polymeric and form unsaturated aldehydes, which, as reported by Richards and Knowles (1968), probably crosslink protein rapidly as follows :



Hardening time : Hardening time with crosslinking agent showed an effect on drug release. Palmieri (1979) found that in in vitro dissolution studies, increasing hardening time with 37% formaldehyde solution decreased the release of undecenovanillylamide from gelatin-acacia microcapsule. But Polk et al. (1994) found that reaction time with calcium chloride had no effect on the release of albumin from chitosan-alginate. Ritthidej and Tiyaboonchai (1995) found that the

lower glutaraldehyde content with longer hardening time could cause more complete crosslinked membrane than at lower hardening time. The results showed that 0.25 gm glutaraldehyde content gave maximum tightening of the microcapsule membrane.

pH and ionic strength : Coacervation pH was studied by Nixon and Hassan (1980) of thiabendazole gelatin-acacia; McMullen, Newton, and Becker (1982) of pectin-gelatin; Koh and Tucker (1988) of gelatin-carboxymethylcellulose; Ritthidej and Tiyaboonchai (1995) of chitosan-carboxymethylcellulose.

Coacervation is caused to occur by pH and ionic strength adjustment. Complex coacervation generally depends largely on charge neutralization at intermediately pH where both positively charged polymer and negatively charged polymer have appreciable opposite charges. The EEP (electrical equivalence pH) is the pH value at which both polyions carry equal and opposite charges measured by the pH zeta potential. At this point the attracting forces between the oppositely charged components are the highest. The optimum pH for complex coacervation is the pH at which equivalents of oppositely charged molecules are present. Optimum pH and ionic strength give maximum coacervate yield. These results were reported by Burgess, Kwok and Megremis (1991) that optimum pH and ionic strength of albumin-acacia were 3.9 and 10 mM, respectively. At low or high ionic strength values the mixtures were clear as occured with gelatin-acacia (Burgess,1991), gelatin-gelatin (Burgess and Carless,1985), albumin-alginic acid (Singh & Burgess, 1989), and albuin-acacia (Burgess, Kwok, and Megremis, 1991) . In gelatin-acacia complex coacervation (Kondo,1979), the pH value of the solution was decreased below isoelectric point of gelatin to make it be positive charges and neutralize with negative charges of acacia. Moreover, pH concerned with active sites of molecular

structure of polymer to form interpolymer complex. If the active sites on the polymer molecules for the complexation are not greatly affected by steric hindrance, the binding ratio should be altered with a change of pH values in the media. This phenomenon has been observed in the complex formation between chitosan and sodium polyacrylate by Takahashi et al. (1990) and chitosan-sodium hyaluronate by Takayama et al. (1990). And if the structure of polymer is rigid, which active sites on the polymer are affected by steric hindrance, changing pH values will not affect the binding ratio of the complexation. This phenomenon has been observed in the complex formation between chitosan and sodium alginate by Takahashi et al. (1990).

Temperature : Temperature influences interpolymer complex formation. In gelatin-acacia complex coacervation, the temperature of the system when mixed with acacia should be more than 35°c which is gelling point of gelatin and temperature for forming microcapsule wall should be rapidly cooled to 5°c. The size of the pores in the coating may be controlled to some degree by the rate of the gelling process. Rapid cooling tends to form a fine pore size, which reduces escape of core material. The results have been stated by Deasy (1984). Ritthidej and Tiyaboonchai (1995) concluded that processing temperature of chitosan-CMC should not exceed 15°c. It was suspected that higher processing temperature created high interfacial energy which caused the microcapsules to coalesce in an attempt to reduce the total interfacial energy of the system.

Stirring rate : Burgess & Carless (1985) stated that increase in shearing forces usually resulted in the production of smaller coacervate emulsion droplets and therefore smaller microcapsules. Nixon and Hassen (1980) showed that the faster stirring speed produced smaller coacervate droplets and smaller range of size

distribution of gelatin-acacia complex coacervated microcapsules of thiabendazole. However, the mean particle diameters and size distributions of albumin-acacia coacervates prepared by Burgess and Singh (1993) at different stirring speeds did not vary significantly. Presumably, faster stirrer speeds would produce small coacervate droplets, which would entrap a drug particle without appreciable tendency to aggregate. At slower stirrer speeds, presumably, larger coacervate droplets would entrap multiple core particles and would tend to be in contact with one another for longer periods of time thus enhancing aggregation and the formation of larger microcapsules.

Feed rate : Productions of microcapsules are mostly produced by stirring the colloid polymer. But there are other techniques which drop or spray one colloid into another by using air-atomization, for example, polylysine-alginate microcapsule wall prepared by Kwok, Groves, and Burgess (1991) and chitosan-CMC microcapsule wall by Ritthidej and Tiyaboonchai (1995); air-jet generator to make chitosan-alginate microcapsule wall by Polk et al. (1994); and vibration nozzle method by Shimano et al. (1995) to make microcapsules of uniformed size. The particle size ranges obtained by these methods depend on the air pressure, distance between the orifice of the atomizer and the pan, size of orifice and the rate of flow from the infusion pump.

Release of drug from microcapsules : (Deasy, 1984)

Release of drug from microcapsules is a mass transport phenomenon involving diffusion of drug molecules from a region of high concentration in the dosage form to a region of low concentration in the surrounding environment. Drug molecules are transported through one or more polymeric membranes comprising

the coating material which normally acts as a barrier. Decreasing in particle size causes increasing the core coating interfacial area, hence the flux of drug passing through microcapsule coating will increase. The accompanying decrease in coating thickness in such a situation would also increase the flux of drug released. The flux of drug passing through the pores of certain microcapsules can be a major pathway of drug release. However, when very fine pores are present in the coating whose diameter is only slightly greater than that of the diffusing species, appreciable resistance is offered by the coating to mass transport. The magnitude of the different concentration, which is taken as the driving force for drug transport across the membrane, will tend to decrease as the solubility of the drug on the upstream side of the membrane decreases. For this reason the dissolution rate of poorly soluble drugs can be a very important factor in limiting drug release from microcapsules.

Many microcapsules, particularly those produced by various coacervation procedures, are multinuclear or are composed of aggregates of smaller microcapsules so that their release kinetics do not follow that expected of a reservoir-type device but rather that of a monolithic device. This is due to diversity of inclusion and lack of homogeneity of many polymeric coatings. Madan (1980) and many others reported that drug release from microcapsules produced by coacervation was proportional to the square root of time, known as Higuchi model.

$$Q = k_1 t^{1/2} \qquad \text{..... Eq. 1}$$

Q = drug mass release per unit area of surface

k_1 = diffusion rate constant

t = time

In summary, it may be stated that drug release rate from microcapsules conforming to reservoir device is zero-order, provided that constant thermodynamic activity is maintained immediately inside the coating material. Microcapsules confirming to monolithic devices and containing dissolved drug release rates that are $t^{1/2}$-dependent for the first half of the total drug release and thereafter decline exponentially. However, if the monolithic device contains a large excess of dispersed drug, the release rate is essentially $t^{1/2}$-dependent thoughout almost the entire drug release. Figure 1 shows the typical form of some of these release rates.

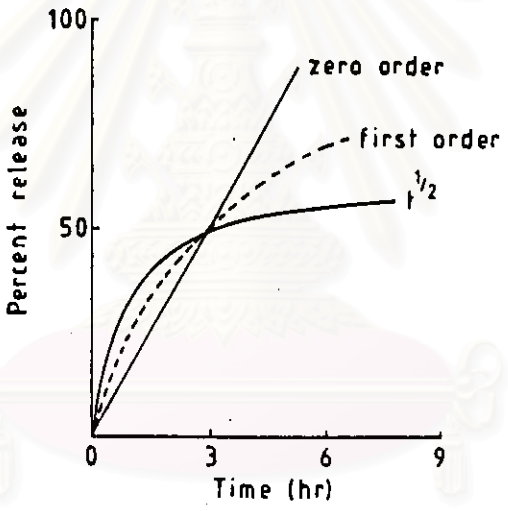


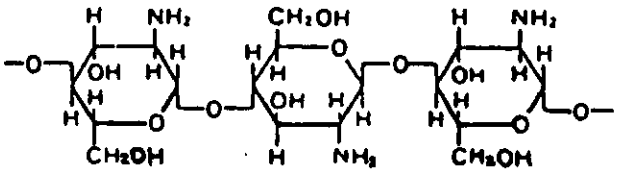
Figure 1 : Zero-order, first-order, and $t^{1/2}$-dependent release for a drug with a half-life of 3 hr.

Chitosan (Lower, 1984; Skaugrud, 1989; Karlsen and Skaugrud, 1991)

Chemical name :

Chitosan : [(1->4)-2-amino-2-deoxy-β-D-glucose]

Structural formula :



(Molecular weight 10,000 - 1,000,000 Dalton.

It depends on its processing conditions.)

Chitosan is a natural hydrophilic cationic polysaccharide prepared from chitin by N-acetylation with alkali. Chitosan is biodegradable and non-toxic.

Chitin and chitosan, similar to cellulose, are long linear chained molecules of (1-4) linked glycan. The repeating unit in chitin is 2-acetamido-2-deoxy-D-glucose (N-acetylglucosamine), while chitosan is an inhomogeneous mixture with the deacetylated form (glucosamine). The mole fraction of deacetylated units (glucosamine), defined as the degree of deacetylation, usually ranges from 70-90%.

Physicochemical properties :

a. Solubility : Chitosan, considered a weak base, will require the addition of acid to solubilize in water and to bring the glucosamine units form $R\text{-}NH_3^+$. Chitosan is soluble in organic acids such as acetic, adipic, citric, formic, lactic, malic, malonic, oxalic, propionic, pyruvic, succinic and tartaric acid. Mineral acids such as hydrochloric, nitric and perchloric acid can solibilize chitosan. But it is insoluble in sulphuric and phosphoric acid.

b. Rheological property : In acid solutions, chitosan can be hydrated and uncoiled the chain branched structure of the molecule. So, chitosan is an excellent viscosifier in an acid environment due to the high molecular weight and the linear unbranched structure. It behaves as a pseudoplastic material showing decresaing viscosity at increased shear. At higher electrolyte concentrations and excess acid a salting out effect occurs, precipitating the chitosan from the solution. Table 2 shows viscosities of chitosan at 1 % concentration in various organic acids.

Table 2 : Viscosity readings for 1 % chitosan on various organic acids at different concentrations.

	Viscosity / pH	
Acid	**10 % acid**	**1 % acid**
acetic acid	260 cps. / 4.1	260 cps. / 3.3
adipic acid	190 cps. / 4.1	
citric acid	35 cps. / 3.0	195 cps. / 2.3
formic acid	240 cps. / 2.6	185 cps. / 2.0
lactic acid	235 cps. / 3.3	235 cps. / 2.7
malic acid	180 cps. / 3.3	205 cps. / 2.3
malonic acid	195 cps. / 2.5	
oxalic acid	12 cps. / 1.8	100 cps. / 1.1
propionic acid	260 cps. / 4.3	
pyruvic acid	225 cps. / 2.1	
succinic acid	180 cps. / 3.8	
tartaric acid	52 cps. / 2.8	135 cps. / 2.5

c. Compatibility : Chitosan is compatible with organic compounds like cationic dyes, cationic surfactants, cationic starches, quarternary ammonium salts as

well as most cationic and nonionic polymers. Multivalent anions will easily crosslink with chitosan to form gels and precipitates.

d. Crosslinked chitosan : Reacting chitosan with a controlled amount of a multivalent anion, will result in a crosslinking between the chitosan molecules. The network formed has the ability to keep large amounts of water, with some systems holding as much as 95 % or more. This crosslinking can be done in acid, neutral or basic environment, depending on the method applied. Several gelling counterions are available such as pyrophosphate, octapolyphosphate, tripolyphosphate (Shiraishi, Imai, and Otagiri, 1993) , alginate, kappa carrageenan, and octylsulphate.

e. Chelating agent : Metals such as Hg, Cd, Pb, Zn, Ni, Cr, Cu, Fe, Mn, Ag, Au, and Pt are able to form complexation with chitosan.

f. Interpolymer complex formation : Chitosan can react with anionic polymers to form interpolymer complex. Examples of anionic polymers are alginate and polyacrylate (Takahashi et al., 1990), acacia and pectin (Meshali and Gabr, 1993).

Applications :

Chitosan has been reported to have some useful medical, cosmetic and pharmaceutical applications.

1. Medical applications (Hou et al ., 1985) : Chitosan has been used as an artificial kidney membrane, blood coagulant, wound healing accelarator, dental

products for caries protection, vascular graft in surgery, soft and hard contact lenses. Chitosan also has some therapeutic activities such as antacid, antiulcer activity, and hypocholesterolemic activity.

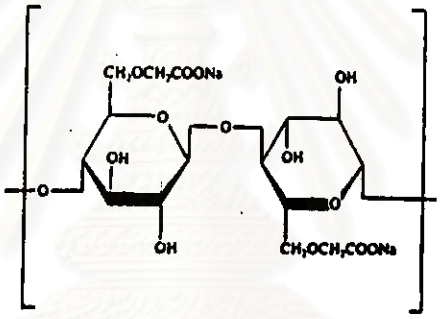
2. Cosmetic applications : Chitosan is used in encapsulation of fragrances, pigments, lotions, hair and skin care products.

3. Pharmaceutical applications : Chitosan has much utilization in the pharmaceutical field. Sawayanagai, Nambu, and Nagai (1982a, 1982b) used chitosan as a vehicle or diluent for direct compressed tablets and ground mixtures. Chitosan has been studied as a tablet binder by Upadrashta, Katikaneni and Nuessle (1992). Shiraishi et al. (1990) used chitosan as an enhancer to increase the disssolution rate of a poorly water-soluble drug by complexing with alginate polymer. Chitosan was used as a vehicle for sustained release preparation by Miyazaki, Ishii, and Nadai (1981). Hou et al. (1985) studied sustained release of indomethacin from chitosan granules of indomethacin in rabbits. Prolonged drug release from matrix formulations of chitosan was investigated by Nigalaye, Adusumilli, and Bolton (1990). Kawashima et al. (1985) prepared controlled release theophylline granules coated with chitosan-sodium polyphosphate. Thacharodi and Rao (1993) prepared crosslinked chitosan membrane and studied the release of nifedipine through the membrane. Chitosan can be used as a film dosage form which was prepared and evaluated by Kanke et al. (1989) and Miyazaki, Yamaguchi, and Takada (1990). Thanoo, et al. (1992) found that chitosan could be used as a suitable matrix of microspheres for the controlled release of pharmaceutical agents. Hassan, Parish, and Gallo (1992) studied chitosan as an agent for preparation of oxantrazole, an anticancer agent, microspheres. Polk et al. (1994) studied albumin chitosan-alginate microcapsules, and Ritthidej and

Tiyaboonchai (1995) studied indomethacin chitosan-CMC microcapsules. Chitosan could be complexed with negatively charged polymer to form complex for a bioadhesive tablet dosage form. This dosage form was prepared and studied of chitosan-sodium hyaluronate by Takayama et al. (1990). For diltiazem chitosan-sodium alginate oral mucosal adhesive tablets were prepared and evaluated by Miyazaki et al. (1995).

Carboxymethylcellulose Sodium (Wade and Weller, 1994)

Structural formula : Structure shown with a degree of substitution (DS) of 1.0



(Molecular weight 90,000-700,000)

Description :

Carboxymethylcellulose sodium (CMC sodium) occurs as a white to almost white color, odorless, granular hygroscopic powder.

Physicochemical properties :

The bulk density of CMC sodium is 0.75 gm/cm^3. Its pK_a is 4.30. CMC sodium is brown at approximately 227°c and char at approximately 252°c. Moisture content of CMC sodium contains less than 10% of water. CMC sodium is

practically insoluble in acetone, ethanol, ether and toluene. It is easily dispersed in water at all temperatures, forming clear colloidal solutions. CMC sodium is incompatible with strongly acidic solutions and with the soluble salts of iron and some other metals. CMC sodium also forms complex coacervates with gelatin and pectin. The viscosities of varoius grades of CMC sodium are shown in Table 3.

Table 3 : Viscosities of aqueous CMC sodium solution at 25°c

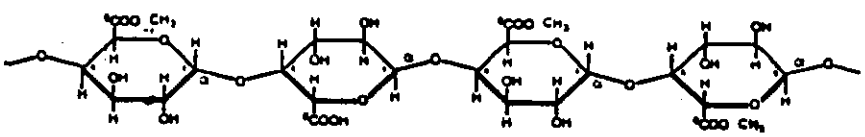
grade	concentration (%w/v)	viscosity (mPaS or cP)
low viscosity	4	50-200
medium viscosity	2	400-800
high viscosity	1	1500-3000

Application :

Carboxymethylcellulose sodium can be used as coating agent, tablet and capsule disintegrant, tablet binder, stabilizing agent, suspending agent, viscosity-increasing agent. Carboxymethylcellulose sodium is generally regarded as a non-toxic and non-irritant material.

Pectin (Pedersen, 1980; Bender, 1959; Smith and Montgomery, 1959)

Structural formula : Segment of high-methoxyl pectin,degree of methoxylation75%



(Molecular weight 50,000-180,000)

Pectin is a linear polysaccharide containing D-Galacturonic acid as the principal constituent of the pectin molecule. The polygalacturonic acid is partly esterified with methoxyl groups, and the free acid groups may be partly or fully neutralized with sodium, potassium, or ammonium ions. The ratio of methoxylated galacturonic acid groups to total galacturonic acid groups is termed the degree of methoxylation (DM).

Type of pectin :

Commercial pectins can be divided by the DM of 50% into high-methoxyl (HM) pectins and low-methoxyl (LM) pectins. These two groups of pectins gelled by different mechanism are shown in Figure 2. HM pectins require a minimum amount of soluble solids and a pH within a pretty narrow range around 3.0 in order to form gels. LM pectins require the presence of a controlled amount of calcium ions for gelation, and do not require sugar and/or acid. The highest DM that can be achieved by extraction of natural raw material is about 75%, yielding a so-called rapid-set , high-methoxyl pectin.

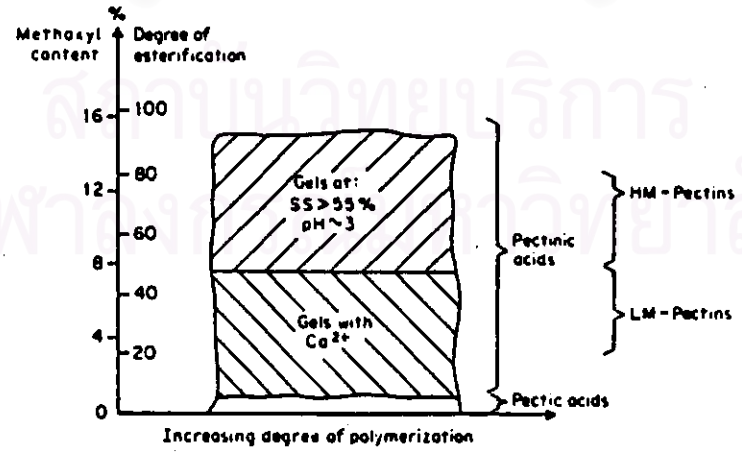


Figure 2 : Nomenclature of pectic substances as related to degree of polymerization and degree of esterification (methoxyl content).

Description :

Color of powdered pectins varies from off white for some citrus pectins to light brown for apple pectins. Pectins have a slightly acidulous taste, but may taste slightly sweet if standardized with sucrose or dextrose, for example, rapid-set citrus pectin containing 5% of saccharin.

Physicochemical properties :

Pectins are soluble in water and insoluble in organic solvents such as alcohols, ethers and hydrocarbons. Pectin solutions are opaque. Equilibrium moisture content of a typical grade HM pectin will be approximately 12% at 70% relative humidity. Pectins react with positively charged macromolecules, e.g., proteins, at pH values below their isoelectric pH. Calcium salts have effect on increasing pectin viscosity. The carboxyl groups of pectin can crosslink with calcium ion.

Applications :

Pectin can be used as gelling agent, thickener, and protective colloid in foods.

Identification : (Smith and Montgomery, 1959)

Precipitation reaction used as basis of proposed procedure for identification of pectin :

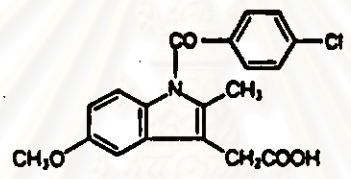
1. pectin + 2/3 vol. 2.5% $CaCl_2$ + 1/3 vol. 3N NH_4OH ⟶ gelatinous precipitate form slowly.

2. pectin + 1 vol. 3N NaOH ⟶ gelatinous flocculent precipitate, yellow colour on heating.

3. pectin + 1/3 vol. basic lead acetate ⟶ translucent gel.

Indomethacin (The United States Pharmacopoeia , 1995 ; McEvoy, 1994)

Structural formula :



(Molecular weight 357.80)

Description :

Indomethacin, an indole acetic acid derivative, is a nonsteroidal anti-inflammatory agent (NSAID). Indomethacin occurs as a pale-yellow to yellow-tan, crystalline powder with a slight odor and is defined as the Form I crystalline, non-solvated free acid moiety of the compound.

Physicochemical properties :

Indomethacin is practically insoluble in water and sparingly soluble in alcohol. The drug has a pK_a of 4.5 for the carboxyl group.. Ultraviolet absorbance

maximum of indomethacin in methanolic 0.1 N hydrochloric acid at 0.00143% concentration is at 318 nm with E_1^1 value of 182. Differential thermal analysis (DTA) peak temperature of indomethacin is 162°c. Indomethacin is sensitive to light and unstable in alkaline solution.

Pharmacology : (O' Brien, McCauley, and Cohen, 1984 ; Flower, Moncada, and Vane, 1990)

Indomethacin has pharmacologic actions similar to those of other NSAIDs. The drug exhibits anti-inflammatory, analgesic, and antipyretic activity. The actions appear to be associated principally with inhibition of prostaglandin synthesis.

Uses : (O'Brien, et al., 1984 ; Flower, et al., 1990)

Indomethacin is used orally or rectally for anti-inflammatory and analgesic effects in the symptomatic treatment of active stages of moderate to severe rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, acute gouty arthritis and acute painful shoulder.

Cautions : (O' Brien, et al., 1984)

Adverse effects have been estimated to occur in 30-60% of patients treated with indomethacin. Gastrointestinal disturbances most frequently reported with indomethacin therapy including nausea, with or without vomiting, and dyspepsia. The oral LD_{50} of indomethacin based on 14-day mortality, is 50 and 12 mg/kg in mice and rats respectively.

Dosage forms :

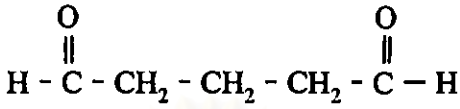
Indomethacin dosage forms placed in the market are capsules, extended-release capsules, suspension, suppositories, and injection (The United States Pharmacopoeia, 1995). The USP drug release specification for Indomethacin Extended-release Capsules is shown in Table 4. There are many studies of indomethacin dosage forms for sustained release, for example, indomethacin chitosan granules were studied by Hou et al. (1985) and Miyazyki et al. (1988). Indomethacin chitosan dried gel was examined by Miyazaki, Ishii, and Nadai (1981). Chitosan-sodium tripolyphosphate gel beads containing indomethacin were prepared by Shiraishi, Imai, and Otagiri (1993). Indomethacin hydrophilic polymer beads consisting of the copolymer of 2-hydroxyethylmethacrylate and acrylamide as the core matrix and ethylcellulose as the barrier membrane were prepared by Surykusuma and Jun (1984).

Table 4 : USP drug release specification Test I for Indomethacin Extended-release Capsules at the times.

Time (hours)	Amount dissolved
1	between 10% and 25%
2	between 20% and 40%
4	between 35% and 55%
6	between 35% and 55%
12	between 60% and 80%
24	not less than 80%

Glutaraldehyde (Gennaro, 1990)

Sturctural formula :



(Molecular weight 100.12)

Description :

Glutaraldehyde is colorless liquid with a pungent odor. It boils at about 188°c with decomposition. Glutaraldehyde is stable in light, oxidizes in air, and polymerizes on heating. Glutaraldehyde is soluble in water and in alcohol.

Applications :

Glutaraldehyde is a disinfectant superior to formaldehyde. It is microbicidal against all microorganisms, including spores and viruses. It uses in sterilization of endoscopic instruments, thermometers, rubber or plastic equipment which cannot be heat sterilized ; as a tanning agent for leather.

Caution : (Budavari, 1989)

Glutaraldehyde causes severe eye irritation in rabbits. LD_{50} of 25% solution orally in rats is 2.38 ml/kg and by skin penetration in rabbits is 2.56 ml/kg.

Identification of glutaraldehyde :

(Inorganic Chemistry Laboratory Handbook of 2nd year Pharmaceutical Faculty, Chulalongkorn University)

1. Schiff 's reagent (Fuchsin aldehyde reagent)

It is the reagent that gives positive test specially for aldehyde, not for α-hydroxyketone. The reaction will happen when the fuchsin, pink colour reacts with sulfur dioxide or H_2SO_3 and changes to be discolour, and then react with aldehyde to form purple complex.

2. 2,4-dinitrophenylhydrazine

It can react with glutaraldehyde to form yellow or orange precipitate. The precipitate can be treated with other solvent to form crystallization of 2,4-dinitrophenylhydrazone which can be melt between 185°-195°c.