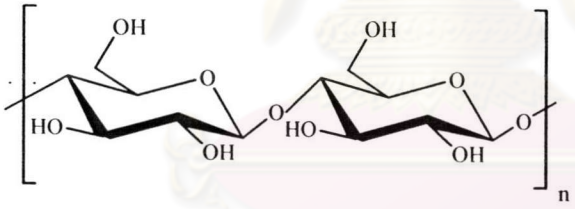
CHAPTER I

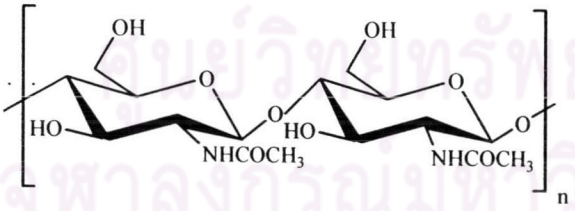
INTRODUCTION

Chitin-Chitosan

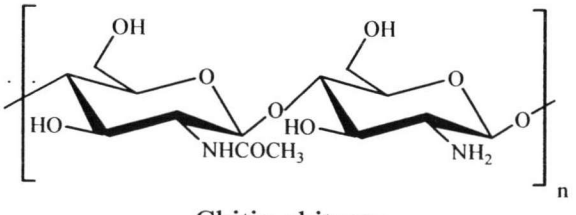
Chitin-chitosan is natural polysaccharide with structure similar to cellulose. Chitosan is obtained from deacetylation of chitin, a naturally occurring structural polymer of crab and shrimp shells. Chitosan is also found in some microorganisms such as yeast and fungi.[1] Chitosan, or partially N-deacetylated chitin, is a linear chain cationic polysaccharide consisting of two monosaccharides, N-acetyl-D-glucosamine and D-glucosamine, joining together by β-(1, 4)-glycosidic linkage. The greater the extent of deacetylation is, the smaller is the proportion of N-acetyl-D-glucosamine in the polymer chain. Since chitin and chitosan are obtained from crab and shrimp shells which are the waste products of Thailand's marine food industry and the polymers can be manufactured and purified in large scale, any researches attempt to increase the applicability of chitosan are highly attractive.



Cellulose



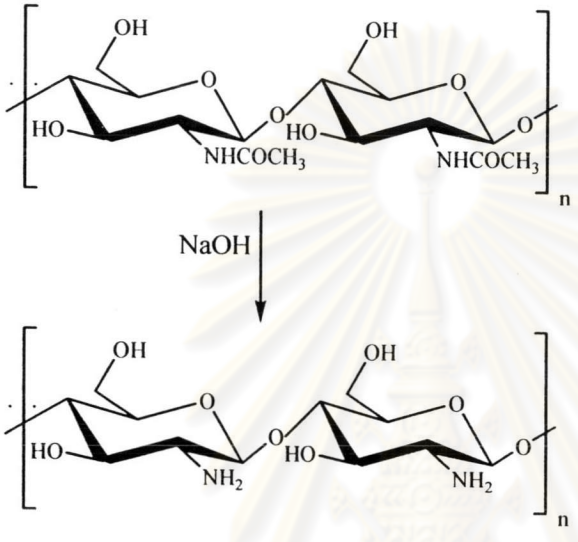
Chitin



Chitin-chitosan

Figure 1.1 Chemical structures of cellulose and chitin-chitosan

Acetamide group of chitin can be converted to amino group to give chitosan. This can be carried out by treating chitin with concentrated alkali solution (Scheme 1). Chitin-chitosan represents long-chain polymers having molecular weight up to several million Daltons. Chitosan can be produced or fabricated into various forms such as powder, film and fiber.[2]



Scheme 1 Deacetylaion of chitin

Chitosan is a natural organic compound, insoluble in water and general organic solvents. However, chitosan can be dissolved in various organic acids, and can be formed into gel, granule, fiber and surface coating.

In nature, the roles of chitin are to protect and make strength to lives' cell-wall. We can found that this polymer has many profits for plants, animals, and human. Natural functions of chitosan include:

1. Activate reaction for cell's working
2. Enhance immunity
3. Disinfect bacteria and mold

Applications of chitin-chitosan span in various industrial fields including:

1) Cosmetics; used in shampoo, hair treatment, lotion, face powder, etc

2) Microbe defending products; 0.02% chitosan was able to inhibit the *E-Coli* in food, similar concentration can also inhibit some plant destroyer microbes.

3) Dietic foods; use for decrease cholesterol and weight control.

4) Waste treatment; used as cationic precipitant for waste water. In environment protection field, chitosan can be applied in waste water disposal, protein recycle and water purification.

5) Dermatology: used in bandage for burn and scale.

6) Agrilcultural application; used as soil improvement, root activator, strength booster, pesticides and microbes (bacteria, virus, mold) defender. Chitosan can be applied in feedstuff additive, seed disposal, soil amelioration and fruit refresh-keeping etc.

7) Preventive medicine; improving immune system, activating calls, preventing cancer, decreasing blood fat/blood pressure/ blood sugar, and fighting aging.

8) Functional material; used in membrane material, carrier, sorbent, fiber and medical materials etc.

9) Textile field, used in fabric sorting, healthcare underwear and paper making.

10) Tobacco application; improving taste, inducing non-poisonous and odorless burning, and also used as tobacco slice glue.

Chitosan Chemical Structure

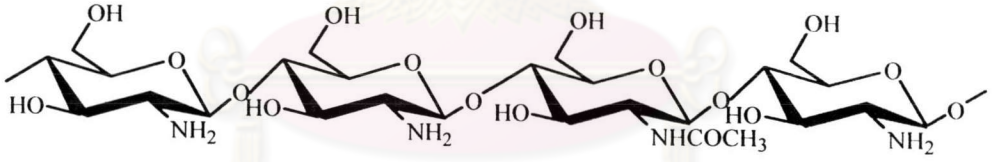


Figure 1.2 Structure of chitin-chitosan

Hydroxyl group

There are two types of hydroxyl groups in chitin-chitosan molecule, primary and secondary. The primary hydroxyl group is more reactive than the secondary one. These hydroxyl groups impose hydrophilicity to chitosan chains and also contribute to the inclusion properties of the polymer.[3] Chitin-chitosan can form inclusion complex with ion or other metals or molecules. Such characteristic is appropriate for industrial wastewater treatment. The lone pair electrons on oxygen atom not only form complex with metal ions such as Ca^{2+}, Ni^{2+}, etc.[4] but also

initiate the microorganism destruction. This latter function of the lonepair electrons induces antimicrobial property to chitin-chitosan.[5]

Amino group

Chitin-chitosan contains reactive primary amino groups those are more reactive than hydroxyl groups. Therefore, chitin-chitosan can be chemically modified. Moreover, the lone pair electron of nitrogen atom can also form bond with ions and metals, bringing the application for wastewater treatment as ion and/or metal absorber to chitin-chitosan.[6] Amino group on the polymer chain can be easily protonated to provide positively charged polymer. The formation of ionic bond between positively charged amino group and negative charge of microorganism cell wall induces antibacterial activity of chitin-chitosan.

Acetamide group

Generally acetamide group is harder to chemically modify than amino group. The acetamide group forms strong hydrogen bond network leading to poor solubility of the polymer in most solvents.

Pyranose ring

Chitin-chitosan consists of pyranose ring either N-acetyl-D-glucosamine or D glucosamine. The research has shown that these rings in the combination with fatty acid can lower cholesterol.[7]

Glycoside linkage

Glycoside linkage (C-O-C) provides biodegradability[8] via enzymatic hydrolysis, i.e., chitosase, chitosanase and lyzozyme. As a result they are responsible for chitin-chitosan chain degradation.

Chemical Modification of Chitosan

For the chemical modification of chitin-chitosan, it is known that chitosan is more practical than chitin because of reactive amino groups. Chitosan can act as a nucleophile to react with other reactive functional groups such as carboxylic acid, acid chloride, and alkyl halide. Chitosan derivatives can be prepared via etherification and esterification. It should be noted that the main problem in chitosan reaction is the dissolvation of the polymer in organic system.

Chemical Modification at Amino Groups

Amino group on chitosan, which is primary amine, can act as a nucleophile due to an unshared electron pair on the nitrogen atom.

Chemical Modification at Hydroxyl Groups

As mentioned earlier that there are two kinds of hydroxyl groups on pyranose ring, primary alcohol at C-6 and secondary alcohol at C-3. However, most reactions have been done on the hydroxyl group at C-6, since it is more reactive.

Gamma Radiation Degradation of Chitosan

Radiation degradation of chitosan is well known due to its effectiveness and ease to control a process. For the past few years, several efforts for low molecular weight chitosan and/or oligochitosan have been done, for example, acid or base hydrolysis[9] and enzymatic digested chitosan.[10]

Many researches on the effect of γ-ray on chitosan properties such as molecular weight and formation of new functional group, have been carried out.[11]

Two steps process has been adopted for chitosan depolymerization for market trial production scale in Thailand. The first step is solid powder irradiation at dose of 100 kGy to reduce M.W. to about $2\text{-}2.5\times10^5$, the second step is 10% solution in 2.5% HOAc irradiation to further reduce M.W. down to oligochitosan (repeating unit, n is 7-14). Collection of water soluble chitosan (monomer to hexamer) can also be done by dissolving the irradiated solid powder in water and succesively precipitate the chitosan by MeOH. The yield is rather small about 0.65% but it is a kind of by product from the process. Low M.W. chitosan has a wide application as fungicide. It has been put to good use for salak (Salacca wallichiana) fruit coating to eliminate some fungi on the skin as well as to preserve its texture by reduce respiration and reduce water evaporation. Oligochitosan solution has been widely used as plant growth promoter[12] in variety of decorative plants, flowering plants, vegetable, and tissue culture protocorm like body, hydroponics, grape and others. Oligochitosan has been mixed with shrimp feed to bond the granule, to reduce bacteria attack and to enhance shrimp growth. Water soluble chitosan may find applications via its antimicrobial activity such as addition into hydrogel wound dresser etc. Its activity of anti-tumor may be a great potential to develop the production process of this functional material to a larger scale.

Sunscreen

The sun is an immense nuclear reactor. As well as producing heat and light, it also emits other types of electromagnetic radiation. Fortunately, the Earth's atmosphere filters out much of the more dangerous solar radiation. We, therefore, cannot detect much of this radiation without special instruments. Beside visible and infrared radiation, some harmful ultraviolet radiation, however, can reach the earth's surface.

Wavelengths of UV Radiation[13]

UV rays have wavelengths shorter than visible light. There are three types of UV rays, grouped by wavelength from longest to shortest, UVA, UVB, and UVC.

UVA is ultraviolet radiation with wavelengths from 320-400 nm. It passes right through the Earth's ozone layer. UVA can cause early aging of the skin.

UVB is ultraviolet radiation with wavelengths of 280-320nm. It does not go as deeply into the skin as UVA does. However, UVB causes skin cancer and other damages. It might also be involved with cataracts. The ozone layer absorbs most of the sun's UVB, but even then the small amount of UVB rays can do substantial damage. With the possibility of the thinning of the ozone layer, more UVB rays should cause more damages.

UVC is ultraviolet radiation with wavelengths shorter than 280 nm. Exposure to UVC is lethal to all organisms. This radiation, however, is completely absorbed by the Earth's ozone layer.

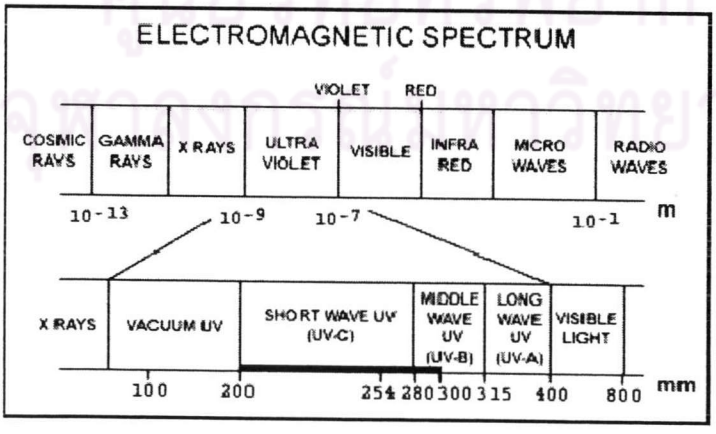


Figure 1.3 Electromagnetic spectrum

The ultraviolet radiation in sunlight and damage[13,14]

Ultraviolet radiation doesn't get very far into bodies. It mainly affects our skin and eyes. The most serious effect of sunlight on skin is the generation of cancer. There are three main types of skin cancer, classified according to the type of skin cell affected. The majority of skin cancers are not lethal. There are cell types in the skin that can become cancerous as a result of exposure to ultraviolet radiation: basal cells, squamous cells and melanocytes. All three occur in the epidermis – the upper layer of the skin.

Sunlight also causes other damage. It can also cause painful sunburn, which usually shows up the day after exposure. Sunlight also ages skin. Sun-weathered skin becomes leathery and loses its softness and luster. Other blemishes can also form. These are unsightly but they are not usually considered dangerous. The eyes are also affected by radiation from the sun. Minor damage will show up in the formation of apterygial, which can be removed.

Sunscreen Chemicals Characters[14]

The use of sunscreen has been a normal practice. There are also a number of UV filters in the market. Choosing of UV filters for particular formulation depends on many factors.

Absorption Range

Good sunscreen chemicals have to absorb the radiation in the range which harmful to human. Practically a choice is made between one or more sunscreens. As a result, a certain bandwidth filter usually corresponds to a combination of UVB and UVA sunscreens. This is a result of an absent of effective broadband UV filter in the market.

Solubility

Whether a sunscreen is based on oil or aqueous gel, the chosen sunscreen compound must be compatible with its base. As a result, a compound that can be miscible with various oils or solvents is easy to use. Water soluble sunscreen is not popular because of the problem on skin persistence.

Sun Protection Factor (SPF)

SPF is a ratio of the amount of UV radiation that produce erythema in skin covered by sunscreen to the moment of UVB radiation that produce a similar

erythema in skin uncovered by sunscreen. The SPF value corresponds to the ability of sunscreen to protect against UV radiation.

Classification of Sunscreen Chemicals[13]

Sunscreen chemicals may be classified according to the type of protections, either as physical blockers or chemical absorbers.

Physical Blocker

A sunscreen that scatters or reflects UV radiation from the skin is called a physical blocker. Zinc cream, which contains zinc oxide, is this type of blocker but is generally only used on small areas of skin as it also prevents heat loss and perspiration from the skin. Titanium dioxide is also used in sunscreens due to its reflective properties. The physical blockers tend to mainly reflect UV radiation; however, they can also absorb UV radiation at specific wavelengths. The sun protection factor (SPF) rating indicates the level of protection provided by a sunscreen against UV radiation.

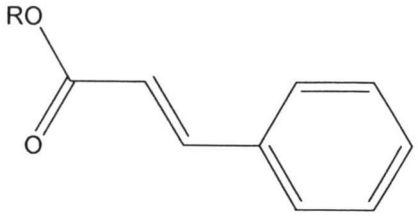
There are, however, still many debates on the safety of physical blockers. Titanium dioxide (TiO_2) has been reported to produce hydroxyl radical under UVA irradiation and, therefore, can induce cytotoxicity.[15] Since TiO_2 exhibits semiconductor properties, they have been used as photocatalyst[16] for the degradation of organic pollutants in waste water. Some investigators have shown that photoexcited titanium dioxide can cause cell death both *in vitro* and *in vivo*.[17]

Chemical Absorbers

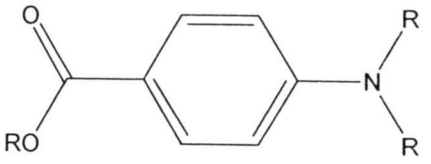
Chemicals absorbers are organic molecules whose absorption bands are in UV region and help absorb the harmful UV radiation.

UVA absorbers are chemicals those absorb radiation in the 320-360 nm region of the UV spectrum. Examples of UVA absorbers include benzophenone, anthranilate, and dibenzoyl methane (see Figure 1.4 for their structures).

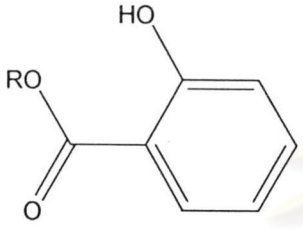
UVB absorbers are chemicals those absorb radiation in the 280-320 nm region of the UV spectrum. Examples of UVB absorbers include *p*-aminobenzoate (PABA) derivatives, salicylate, cinnamate and camphor derivatives (see Figure 1.4 for their structures).



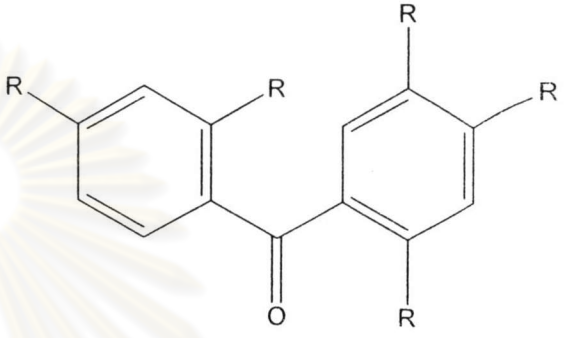
A. Cinnamate derivatives



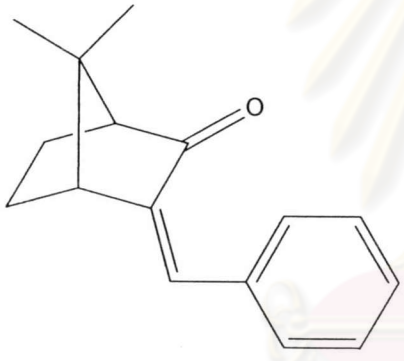
B. *p*-aminobenzoate (PABA) derivatives



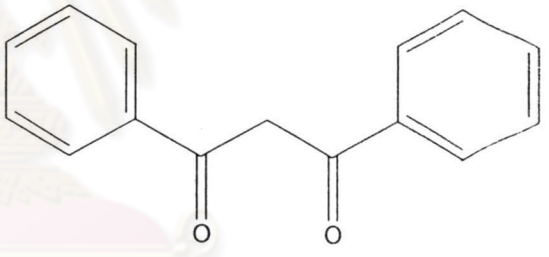
C. Salicylate



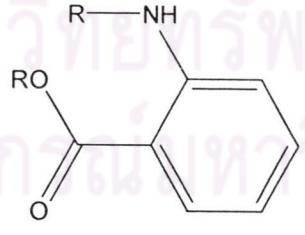
D. Benzophenone



E. camphor derivatives



F. Dibenzoylmethane



G. Anthranilate

Figure 1.4 The sunscreen absorbers used in the sunscreen industry

Mechanism of Sunscreen Action[18]

Sunscreen chemicals are generally aromatic compounds conjugated with a carbonyl group. An electron releasing group such as amine or methoxy group is substituted in the ortho or para position of the aromatic ring. They absorb the harmful short-wave (high energy) UV rays (280-400 nm) and convert the remaining energy into longer wave (low energy) radiation. The process of sunscreen action is called "photophysical process" which no change in molecular structure. Mechanism of sunscreen action (Figure 1.5) involves excitation of electron from ground state by absorbing the UV rays. Then upon the return of electron from excited state to ground state, the absorbed energy is emitted in the form of IR or heat.

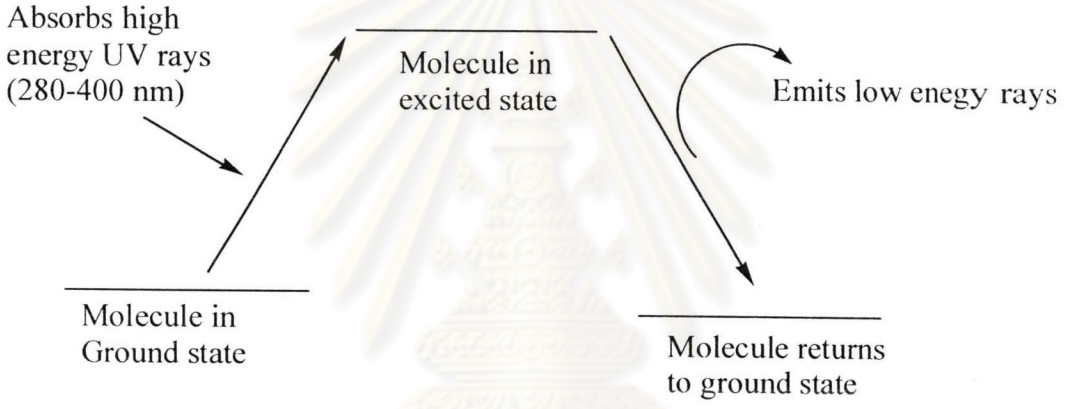


Figure 1.5 Mechanism of sunscreen action

Literature Reviews

Chitosan

Derivatization of chitosan

As can be analyzed from the chemical structure, chitosan exhibits high crystallinity though inter- and intramolecular hydrogen bond network. Combining with the high molecular weight, chitosan possesses problems on solubility and reactivity. The polymer is quite inert and can be dissolved only in acids. However, chitosan is attractive to propose novel derivatives.

In 1980, Fujii *et al.*[19] illustrated that the derivative achieved from the complete N,O-polyacylation of chitosan with an excess of long-chain acid chloride can be soluble in some organic solvents such as N,N-dimethylacetamide, dimethylsulfoxide and pyridine.

In 1991, Nishimura *et al.*[20] studied availability of regioselective chemical modifications in homogeneous solution under mild conditions for efficient transformations of chitosan into variety of soluble derivatives by using completely N-protected derivative. Selective and quantitative N-phthaloylation of chitosan proceeded smoothly by the reaction of chitosan with phthalic anhydride in N,N-dimethylformamide (DMF) at 130°C. The resulting phthaloylchitosan exhibited much improved solubility in common organic solvents such as DMF, N,N-dimethylacetamide, dimethylsulfoxide and pyridine.

In 2001, Kurita *et al.*[21] studied chemoselective N-phthaloyl of chitosan which could be accomplished successfully in one step using DMF containing 5% water as solvent. Crystallinity of N-phthloylchitosan was observed. The N-phthloylchitosan exhibited high affinity for organic solvents, although somewhat lower than that of N-phthaloylchitosan with additional O-phthaloyl groups.

In 2003, Yoksan *et al.*[22] studied controlled hydrophobic/ hydrophilicity of chitosan for spheres without specific processing technique. Chitosan was functionalized with phthalic anhydride at the amino group and poly (ethylene glycol) methyl ether at the hydroxyl group via homogeneous reaction. The product could form spherical with the size of few micrometers.

In 2003, Zhang *et al.*[23] reported the new method to introduce O-succinyl group into chitosan under the protection of amino group. Protection group at the amino moieties was removed lastly by using hydrazine hydrate. O-succinyl-chitosan showed much higher solubility in water. The study of enzymatic degradation revealed that the O-succinyl-chitosan was of low susceptibility to lysozyme. Improving water solubility of the polymer by attachment of succinyl group into through thr hydroxyl functionality marks the significance of this study. The change of chitosan structure decreases the intermolecular hydrogen bonds form thus damages the formation of crystallization. The obtained product can, therefore, be further chemically modified and may have potential biomedical applications.

In 2005, Lebouc *et al.*[24] studied two ways of grafting PEG onto chitosan. According to the way used, characteristics of PEG-g-chitosan were different. In the first way, primary alcohol on chitosan was first protected before PEG was introduced. The second way allowed PEG grafting onto original chitosan directly.

Irradiated Chitosan

For the past few years, several efforts to produce low molecular weight chitosan and/or oligochitosan have been done, for example, acid or base hydrolysis[9], enzymatic digestion and radiation degradation.

In 1996, Andrady *et al.*[25] studied UV irradiation on chitosan and found that acetamide groups were cleaved and changed to amino side groups when using low energy (λ>360 nm), while glucosidic linkages were broken at high energy level (λ>260 nm) to form carbonyl at the chain end. In addition, the decrease of molecular weight was directly propotional to the energy use.

The γ-ray radiation process can be carried out in both dry and wet states. There are many researchers studied on the effect of γ-ray on chitosan properties such as the decreasing of molecular weight in various conditions and the formation of new functional group.

In 1988, Aebi *et al.*[26] studied the γ-ray irradiation on the wet state of chitosan, i.e., aqueous solution or water-swollen film. The molecular weight was found to decrease for 10 times with dose 1.0 Mrad for wet state whereas irradiation for dry state showed the decrease in viscosity 13.4 times with the same dose.

In 1992, Ulanzki[27] examined the changes of chitosan structure induced by radiation. It was concluded that the radiation yielded the chain scission in solid state at 0.9 mol/J in vacuum, 1.1 mol/J in air, and 1.3 mol/J in oxygen while the corresponding yielded crosslinking was equal to zero. The studies also classified the molecular weight decreases and the carbonyl and carboxyl groups were formed as the γ-ray dose increased.

In 2002, Siri-Upathum[28] reported the radiation dose required for preparation of oligochitosan. Mv of 45 kDa was found to be 75 kGy using 10% of 100 kGy irradiated chitosan in solid form in 2.5% HOAc. At this dose, the percentage of oligochitosan with DP>8 was about 30%. Upon using the neutralized oligochitosan solution to investigate for young orchid plant growth promotion.

Sunscreen

On certain aspects, sun light can be beneficial (Vitamin D synthesis). However, it can also have serious damaging on skin such as actinic aging, sunburn, photoaging, and skin cancers of different types.[29] Thus, it is very important to use sunscreen agents in order to protect oneself from the effects of UV. Health agencies

world-wide also recommend the use of sunscreens as a means of lowering the risk of developing skin cancer.

Recently, destruction of the ozone layer that acts as a barrier layer protecting against ultraviolet rays from the sun has been occurring along with the progression of global scale environmental changes. As a result, the bare skin of people has become exposed to stronger ultraviolet (UV) rays from the sun. These UV rays include high amounts of energy and have a variety of harmful effects on the skin. Thus, many attentions have focused on the function of UV ray protection agents.

Sunscreens have become the primary means to minimize those possible damaging effects and other photosensitivity and phototoxicity on human skin. Esters of 4-methoxycinnamic acid are among the popular UVB screening compounds used in various cosmetic formulations in sunscreen products. The most widely used derivative in this group is the 2-ethylhexyl-*p*-methoxycinnamate (OMC) which possesses a high molar absorption coefficient (ε), approximately 22,000-24,000 $M^{-1}cm^{-1}$, and shows only few allergic reactions to human skin.[30] Nevertheless, transdemal permeation of the compound into human body has been reported[31] and possible toxicity and phototoxicity of OMC was suspected.[32]

Many researches have indicated transdermal penetration of many other small molecule UV filters.[33]

In 1997, Heyden *et al.*[34] reported that OMC could penetrate through the skin layer into blood circulation. Such conclusion was drawn from the discovery that OMC could be recovered from milk of human volunteers.

In 2000, Benech-Kieffer *et al.*[35] studied the percutaneous absorption of OMC sunscreens in vitro. OMC absorptions were detected in both pig and human. The correlation of the qualitative data between pig skin and human skin was very good. This research confirmed the OMC absorption through both pig's and human's skin.

Existing problem solving method for transdermal absorption of sunscreen included encapsulation of sunscreens into various carriers to slow down their skin penetration rates, making UV filter as a polymer molecules and adjusting formulation to reduce the penetration rate.

In 2003, Jimenez *et al.*[36] reported the evaluation of the *in vitro* transdermal permeation and skin accumulation of octyl methoxycinnamate (OMC) through pig skin. They determined the quantity of OMC in different pig skin layers

(stratum corneum, viable epidermis, dermis, and receptor fluid). The study showed that by encapsulating OMC into nanocapsule, skin permeation of OMC could be decrease.

In 2001, Godwin *et al.*[37] determined the influence of Transcutol® CG concentration on the transdermal permeation and skin accumulation of two ultraviolet (UV) absorbers, 2-hydroxy-4-methoxybenzophenone (oxybenzone) and 2-octyl-4-methoxycinnamate. The results demonstrated that the inclusion of Transcutol® CG in sunscreen formulations increased the skin accumulation of the UV absorbers oxybenzone and cinnamate without a concomitant increase in transdermal permeation.

In 2002, Lorenze[38] studied the treatment and prevention of sunburns by UV light exposure using SPF enhancer, astaxanthin. Prevention of sunburns by using orally administered astaxanthin was tested. These effects in combination or separately were able to retard and prevent sunburns, when astaxanthin was ingested, injected, or delivered by a cream in a therapeutically effective dose.

In 2003, Yener *et al.*[39] prepared solid lipid microspheres (SLM) of octyl methoxy cinnamate (2-ethylhexyl-*p*-methoxycinnamate;OMC). SLM was used as carriers for OMC in order to decrease the release and penetration rate of this UV absorber. Incorporation of OMC into SLM also enhanced the photostability of OMC compared to plain absorber in various vehicles. Another important aspect was the effectiveness of OMC in liposphere form which showed nearly the same protection as the free form after exposure to a solar simulator.

In 2003, Pattanaargson *et al.*[40] grafted 4-methoxy cinnamic acid on silicone. The obtained product, gave UV absorption profile similar to that of OMC but with much lower skin permeation and better photostability property.

In 2004, Dueva *et al.*[41] provided a composition that had enhanced photo-protective properties without the inclusion of additional sunscreen agent. The invention indicated that carotenoid in the presence of sunscreen agent synergistically increased the SPF of the composition compared to a composition without carotenoid.

To overcome the transdermal absorption of sunscreen, here it was hypothesized that covalent links between multiple units of the 4-methoxycinmate moieties and a chitosan polymer would create low-penetrating biocompatible UV-screening compounds. Skin penetration of the 4-methoxycinnamate moieties could be avoided because of the presumably very little diffusion through skin of large polymer

over a limited period of time. Herein it was described the grafting of 4-methoxycinnamiate moieties onto chitosan polymers, the study of the photostability of the grafted products and the investigation on the UV absorption profiles of the products.

Scope and objective of the present work

As mentioned that problem of skin permeation of sunscreen has been realized and previous study by Hongchinakorn has shown that by grafting UV absorptive chromophore, 4-methoxycinnamoyl group, onto silicone polymer, skin permeation of the UV filter could be reduced. This study, therefore, plan to create UV-absorptive chitosan derivatives by grafting some UV-absorptive chromophores such as, 4-methoxycinnamoyl and 2,4,5-trimethoxycinnamoyl groups onto chitosan. We hypotisized that by doing so, solubility property of the obtained UV-absorptive chitosan will be improved. The use of chitosan polymer backbone will make the obtained UV-filter polymer biocompatible and non-toxic.

Grafting of such chromophores could be done through esterification between 4-methoxycinnamic acid (or 2,4,5-trimethoxycinnamic acid) and phthaloylchitosan. Both phthaloylchitosan and 2,4,5-trimethoxycinnamic acid were first prepared as described previously.[42]

Evaluations of products were done on the following aspects:

- Chemical structure characterization
- Solubility
- UV-absorption property
- Photostability