# CHAPTER I

# INTRODUCTION

## 1.1 Chitin-chitosan

Chitosan is a natural aminocopolysaccharide, similar in structure to cellulose. Both are made by linear β-(1→4)-linked monosaccharides. However, an important difference to cellulose is that Chitosan is composed of 2-amino-2-deoxy-β-D-glucan combined with glycosidic linkages. The primary amine groups render special properties that make chitosan very useful in pharmaceutical applications. Compared to many other natural polymers, chitosan has a positive charge and is mucoadhesive [1]. Therefore, it is expected to be used extensively in drug delivery applications [2-6]. Chitosan is obtained from the deacetylation of chitin, a component in exoskeleton of crustaceans and insects as well as in cell wall of yeasts and fungi. Acetamide group of chitin can be converted into amino group to give chitosan and this which is carried out by treating chitin with concentrated alkali solution. (Scheme 1) The structures of cellulose, chitin, chitosan and chitin-chitosan are shown in Figure 1.1.

**Scheme 1.1**: Deacetylation of chitin to chitosan

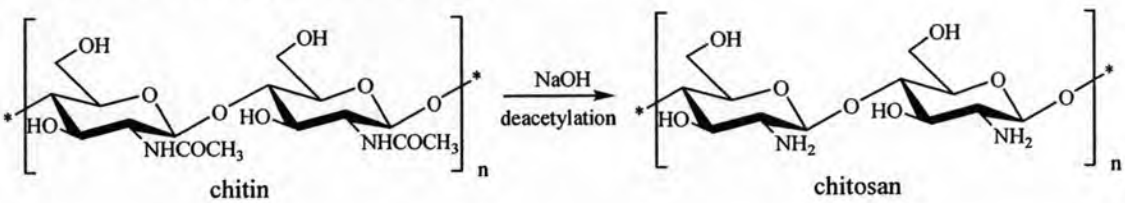
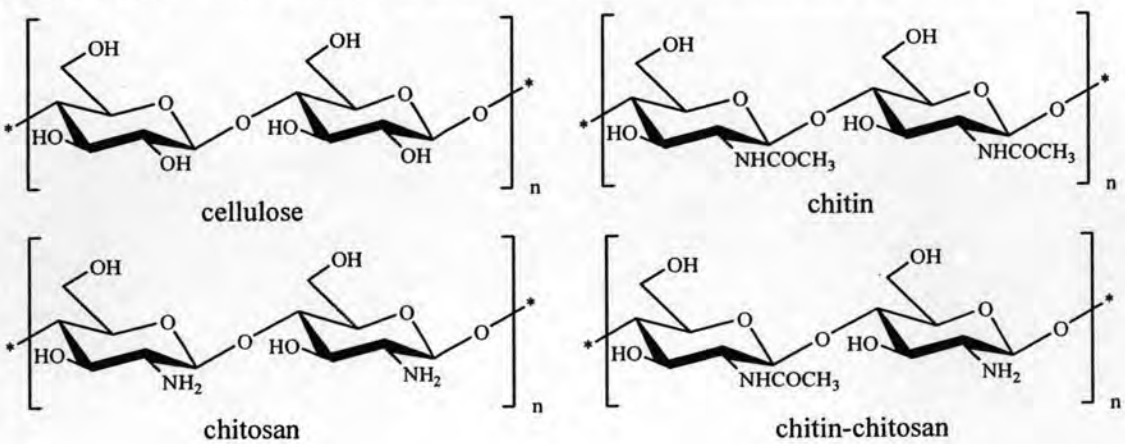



**Figure 1.1**: Chemical structures of cellulose, chitin, chitosan and chitin-chitosan



Chitin-chitosan or chitosan represents long-chain polymers having molecular mass up to several million Daltons. Commercially available chitosan has an average molecular weight ranging between 3,800 and 20,000 Daltons and is 66% to 95% deacetylated.

Chitosan is a cationic polysaccharide in neutral and basic pH conditions. The polymer contains free amino groups and hence, is insoluble in water. In acidic pH, amino groups can undergo protonation thus, making it soluble in water. Solubility of chitosan depends upon the distribution of free amino and N-acetyl groups [7]. Usually 1–3% aqueous acetic acid solutions are used to solubilize chitosan. Chitosan is biocompatible with living tissues since it does not cause allergic reactions and rejection. It breaks down slowly to amino sugars, harmless products, which are completely absorbed by the human body [8]. Chitosan degrades under the action of ferments, it is nontoxic and easily removable from the organism without causing concurrent side reactions [9]. It possesses antimicrobial property and absorbs toxic metals like mercury, cadmium, lead, etc [10]. In addition, it has good adhesion, coagulation ability, and immunostimulating activity [11].

Chitosan has been recently employed in developing micro/nanoparticles. The polymer is a linear polyamine containing a number of free amine groups that are readily available for cross-linking, its cationic nature allows for ionic cross-linking with multivalent anions, it has mucoadhesive character, which increases residual time at the site of absorption, and so on. Chitin and chitosan have very low toxicity. Various sterilization methods such as ionizing radiation, heat, steam and chemical methods can be suitably adopted for sterilization of chitosan in clinical applications. In view of the above-mentioned properties, chitosan is extensively used in developing drug delivery systems [12-14]. Particularly, Chitosan has been used in the preparation of mucoadhesive formulations [15-17], improving the dissolution rate of the poorly soluble drugs [18-19], drug targeting [20, 21] and enhancement of peptide absorption [22].

**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. Up to now, much work has been reported on the chemical modification of chitosan.

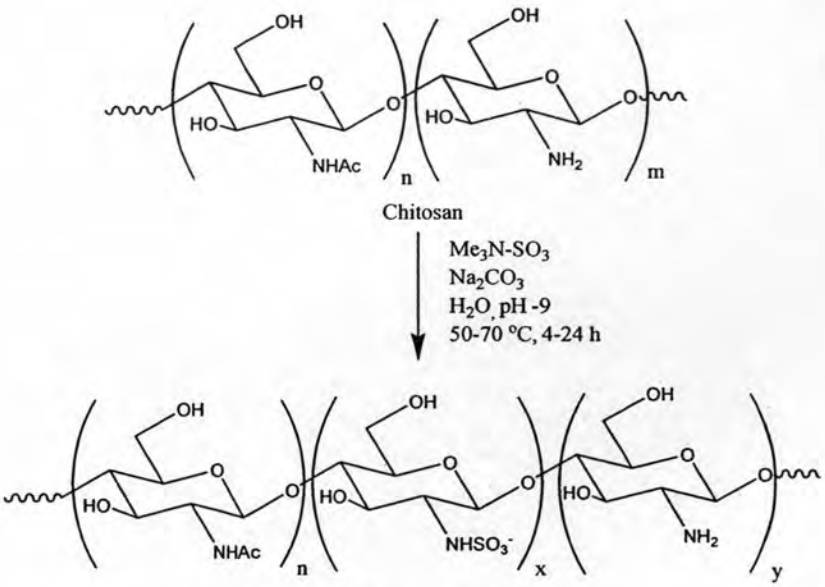
*O-and N-Carboxymethylchitosans*

Carboxymethylchitosan (CM-chitosan) is the most fully explored derivative of chitosan; it is an amphoteric polymer, whose solubility depends on pH. Under controlled reaction conditions (with sodium monochloracetate in the presence of NaOH), one gets O- and N-carboxymethylation. The yield of substituents on the three positions was determined by NMR [23]. This reaction extends the range of pH (pH > 7) in which chitosan is water-soluble, but a phase separation due to the balance between positive and negative charges on the polymer was observed at 2.5<pH<6.5.

Most interesting is the preparation of N-carboxymethylchitosan by reaction with glyoxylic acid in the presence of a reducing agent [24]. The distribution of monosubstituted (–NH–$CH_2COOH$) and disubstituted (–N (–$CH_2COOH)_2$) groups was established by $^1H$ and $^{13}C$ NMR. Disubstitution is easily obtained, giving an interesting derivative for ion complexation. A specific oxidation of the C-6 position hydroxyl group was realized using the TEMPO reactant on chitin to produce a chitin-based hyaluronic acid analog [25]. This derivative is water soluble in a wide range of pH, but only if it is prepared from a fully acetylated chitin.

*Chitosan 6-O-sulfate*

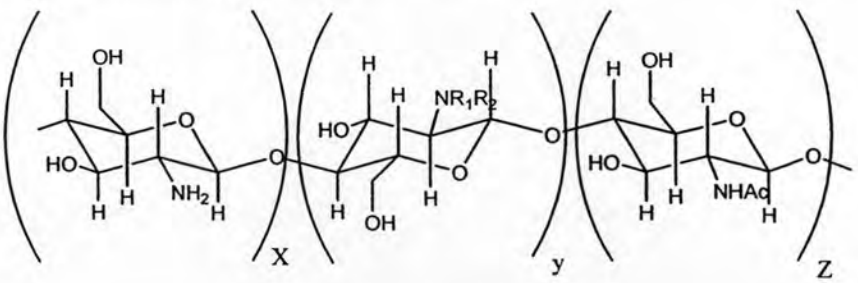
This derivative is an anticoagulant; it was first prepared as an O- sulfated derivative [26] and more recently as N-sulfated chitosan [27].




Synthesis of *N*-sulfated chitosan

*N-methylene phosphonic chitosans*

These interesting anionic derivatives with amphoteric character were synthesized under various conditions and proved to have good complexing efficiency with cations such as $Ca^{2+}$, and those of transition metals (Cu (II), Cd (II), Zn (II) etc.). [28] The complexation provides corrosion protection for metal surfaces [29]. These derivatives were also modified and grafted with alkyl chains to obtain amphiphilic properties that have potential applications in cosmetics [30].



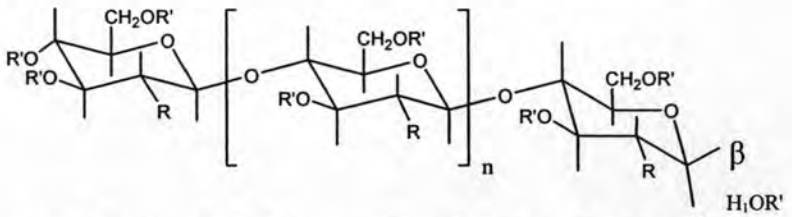

$R_1 = H, R_2 = CH_2PO_3H_2$
$R_2 = R_2 = CH_2PO_3H_2$

Chemical structure of *N*-methylene phospholic chitosan

*Trimethylchitosan ammonium*

This cationic derivative, water soluble over all the practical pH ranges, is obtained by quaternization of chitosan [31] with methyl iodide in sodium hydroxide under controlled conditions, and has been fully characterized by NMR [32]. A large decrease of molecular weight during this reaction is observed under all conditions

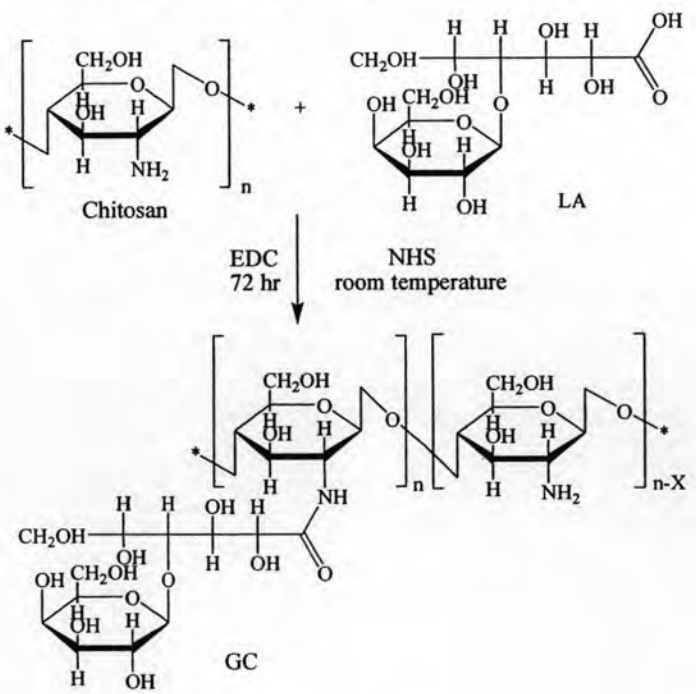
tested. These polymers show good flocculating properties with kaolin dispersions, suggesting applications to paper making [33]. Other quaternized derivatives have been prepared and claimed to have antistatic properties [34].



Chemical structure of *N*-trimethyl chitosan chloride –R = -$NH_2$ and/or –$N^+(CH_3)_3$, Cl-, -R' = -H and/or $CH_3$

*Carbohydrate branched chitosans*

Carbohydrates can be grafted on the chitosan backbone at the C-2 position by reductive alkylation: For that purpose, disaccharides (cellobiose, lactose, etc.) having a reducing end group, are introduced, in the presence of a reductant, onto chitosan in the open-chain form [35]. These derivatives are water soluble. Galactosylated chitosan was an example of this group of chitosan derivative [36]. Carbohydrates can also be introduced without ring opening on the C-6 position [37]. These derivatives are important as they are recognized by the corresponding specific lectins and thus could be used for drug targeting [38]. A special case of chitosan grafted with cyclic oligosaccharide, cyclodextrin is discussed below.

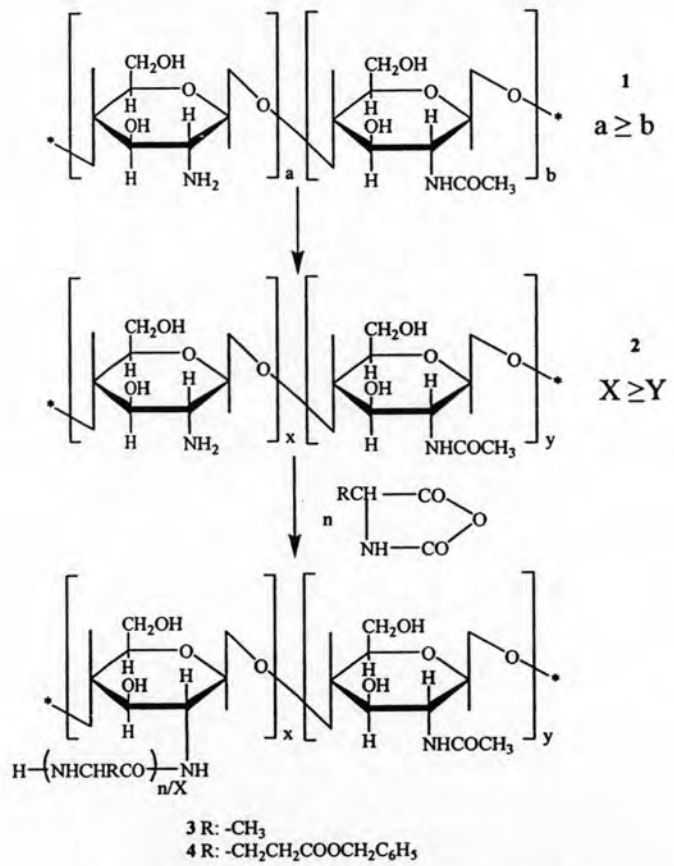



Synthesis of galactosylated chitosan (GC)

*Chitosan-grafted copolymers*

One of the most explored derivatives of chitosan-grafted copolymers is poly(ethylene glycol)-grafted chitosan (PEG-grafted chitosan), which has the advantage of being water soluble, depending on the degree of grafting: higher molecular weight PEG at low DS gives higher solubility than low molecular weight PEG. PEG can be also be introduced by reductive amination of chitosan using PEG-aldehyde [39].

Polypeptides have also been grafted onto chitosan by reaction with N-carboxyanhydrides of amino acids with the purpose of developing new biomaterials [40].

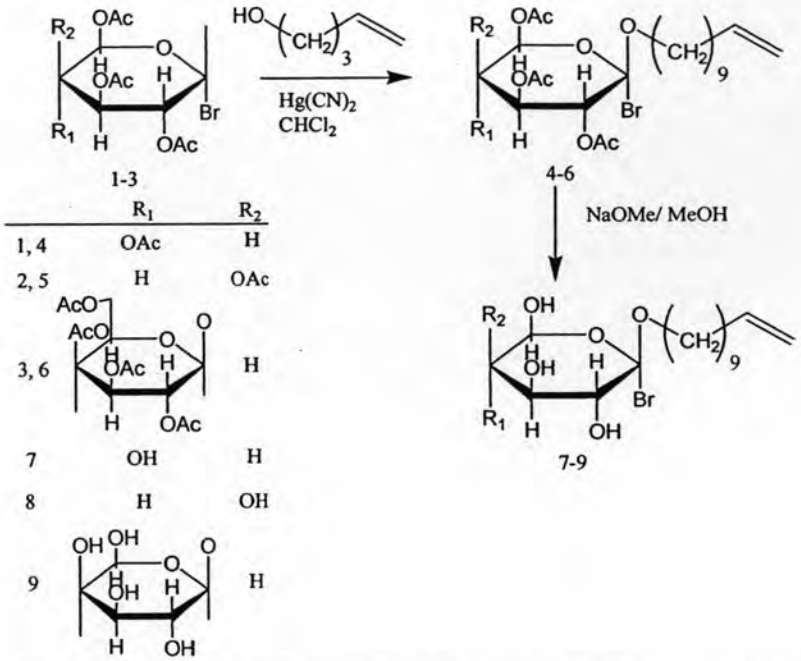



Synthetic route to graft copolymer

*Alkylated chitosans*

Alkylated chitosans are very important as amphiphilic polymers based on polysaccharides. The first derivative having these characteristics was a C-10-alkyl glycoside branched chitosan with a high degree of substitution (DS=1.5), which gelled when heated over 50 °C [41]. Another approach was used for selective N- and O-palmitoylation giving a derivative with two or three long alkyl chains per

monomeric unit. This reaction involved protection and deprotection of the C-6 position [42].




Synthesis of 10'-undecenyl $\beta$-D-glycosides of glucose (7), galactose (8) and lactose (9), respectively

By using carboxylic anhydrides with different chain lengths on CM-chitosan, highly substituted derivatives with low regularity were obtained. They were insoluble in water and their biodegradability was decreased [43].

Using the reductive amination, a series of amphiphilic derivatives were produced with different chain lengths (Cn from 3 to 14) and controlled DS (usually lower than 10% to maintain water solubility in acidic conditions) [44]. This technique was also used to introduce n-lauryl chains [45]. Alkylated chitosans with good solubility in acidic conditions (pH < 6) have a number of very interesting properties. First, they exhibit surface activity [46-49]. Second, they increase considerably the viscosity of aqueous solution due to hydrophobic inter-chain interactions. For example with C-12 chain length and a DS~5%, a physical gel is obtained. It is interesting to mention that alkyl chitosans are compatible with neutral and cationic surfactants; it has been demonstrated that cationic surfactant was adsorbed on the alkyl chains which were grafted on chitosan backbone, thus promoting its solubilization [50].

*Cyclodextrin-linked chitosans*

The cyclic oligosaccharides, namely α-, β-, γ- cyclodextrins (CD), are important because of their ability to encapsulate hydrophobic molecules in their toroidal hydrophobic cavity, whose selectivity depends on the number of glucose units (respectively 6, 7, 8 d-glucose units) [51-53]. For various applications, it is interesting to graft the cyclodextrin on a polymeric backbone such as a biocompatible polysaccharide. A synthesis of α- and β-cyclodextrin-chitosans with relatively high degree of substitution has been described [54]. The authors found that these new derivatives had the ability to differentially recognize and retain certain guest compounds based on their molecular shapes and structures. They proposed to use these polymers as supports for reverse-phase adsorption or as adsorbents in controlled release systems.

**Drug delivery system**

For many years, chitin-chitosan has been reported to be materials suitable for the use as drug carriers in pharmaceutical, medical and biotechnological areas. Drug delivery system (DDS) is an advanced and value-added application for most biodegradable polymers. The DDS offers numerous advantages compares to conventional dosage forms such as increasing the therapeutic activity, reducing the toxicity and the number of drug administrations required during treatment, including the convenient treatment. Considering drug delivery system (DDS), the challenge for high efficiency drug is about drug incorporation and systematic release mechanism.

Colloidal systems have found numerous applications as promising delivery vehicles for drugs, protein, antigens, and genes due to their low toxic side effects and enhanced therapeutic effects. Polymeric self-assembly systems, (SA's) are one type of colloidal system that has been widely investigated in terms of micellar behavior in the areas of biotechnology and pharmaceutics. Precise control of size and structure is a critical design parameter of micellar system for drug delivery applications.

Recently, the self-assembly of chemically modified chitosan into nanoparticles has been investigated for the delivery of macromolecule. This method gives particles without cross-linking agent and complication of chemical reaction. The drug molecules are incorporated into micro- or nanoparticles via non covalent bonds such as hydrophobic/hydrophobic or hydrophilic/hydrophilic interaction to give drugs with a maintained drug active site as well as the high efficiency in controlled release under

the high surface area of particles. Generally, the key factor for sphere formation is self-aggregation of amphiphilic polymeric chains in order to minimize the difference in interfacial free energies in aqueous and/or organic solvents [55].

**Antibacterial activity of chitosan**

Many researchers have continued studies in this field. The mechanism behind this activity can be summarized as follows:

(1) The cationic nature of chitosan causes it to bind with sialic acid in phospholipids, consequently restraining the movement of microbiological substances [56].

(2) Oligomeric chitosan penetrates into the cells of microorganisms and prevents the growth of cells by preventing the transformation of DNA into RNA [57].

However, the water-insolubility of chitosan inhibits its wide application as an antibacterial agent. Jung et al. prepared anionic side-chain-grafted, water-soluble chitosan (WSC) derivatives having zwitterionic properties [58]. To prepare these derivatives, mono(2-methacryloyl oxyethyl) acid phosphate and vinyl sulfonic acid sodium salt were grafted onto chitosan. Antimicrobial activity against Candida albicans (Ca), Trichophyton rubrum (Tr), and Trichophyton violaceum (Tv) depended largely on the amount and type of grafted chains as well as changes of pH. The highest activity was shown at pH 5.75 against Ca and Tv, due to the difference in affinity between cell walls of fungi and the chitosan derivatives.

## 1.2 PEG and PEG Derivatives Used in Cosmetics [59]

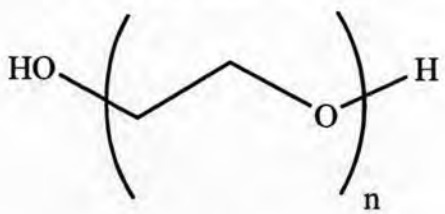



Figure 1.2 Structure of polyethylene glycols

Polyethylene glycols (PEGs) are polymers of ethylene oxide with the generalized formula HO-($CH_2$-$CH_2$-O)$_n$-H in which "n" indicates the average number of oxyethylene groups. The compound has been assigned the scientific name "poly(oxy-1,2-ethanediyl)-α-hydro-ω-hydroxy". PEGs and PEG derivatives do not represent definite chemical entities, but are mixtures of compounds with varying

polymer chain lengths. The average number or the molecular weight of the polymer chain is often indicated in the generic name of the specific substance, as for instance, in PEG-8 which is equivalent to PEG-400. The first nomenclature, i.e. using the average length of the polymer chain, will be used in the following assessment, as it is the common naming convention in the cosmetic industry.

PEGs with mean molecular weights of up to 400 are clear viscous liquids at room temperature. PEGs of higher molecular weights are white waxy solids. All PEGs are readily miscible with water, the solid PEGs are readily miscible with water, the solid PEGs are slightly less soluble in water with their solubility decreasing as molecular weight increases. They are non-volatile, stable compounds, which do not hydrolyse or, in the absence of oxygen, deteriorate on storage.

In cosmetic, the PEGs and their derivatives are widely used as surfactants, cleansing agents, emulsifiers, skin conditioners, and humectants. These compounds are used in cosmetic applications because of their solubility and viscosity properties, and because of their low toxicity [60].

In the pharmaceutical industry, they are used as vehicles for drugs and as ointment bases, capsules, tablet and pill binders, suppositories liquid prescriptions, and in veterinary drugs, including parenteral, topical, ophthalmical, oral, and rectal preparations [61].

### 1.3 Cosmetic Actives

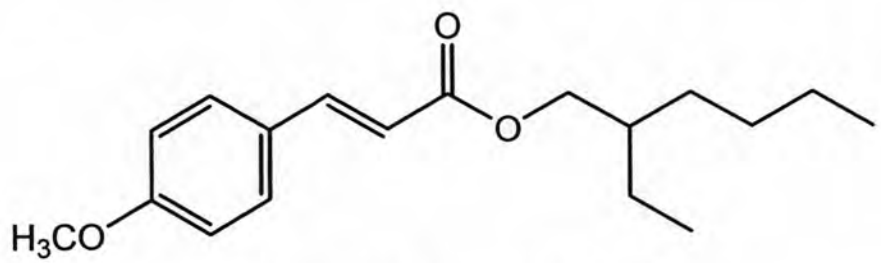
In general, the main purpose of a cosmetic product is to protect the skin and to delay the aging process of the skin. This process is obviously very complicated, and this process is obviously very complicated, and depends on many variables such as exposure to UV light, climatic conditions and dietary habits. For example, UV rays may generate highly reactive free radicals, which through chain reactions can cause irreversible alterations in the cutaneous tissue. These changes can lead to the appearance of wrinkles, which are caused by the breakdown of the collagen pillars of the dermis [62]. In order to minimize the harmful effects of UV light, modern cosmetic products contain essential ingredients such as UV protecting agents that may absorb or reflect the light effectively at the required wavelength. In addition, they may also contain molecules that can trap free radicals. Therefore, a suitable cosmetic product should contain components with specific activity, should deliver these

components to the skin and cause adhesion to the skin in order to achieve the best performance.

Nowadays, there are many cosmetic ingredients for example sunscreening agent such as *trans*-2-ethylhexyl-p-methoxycinnamate (*trans*-EHMC), anti-ageing such as α-tocopheral (vitamin E), ascorbic acid (vitamin C), astaxanthin, coenzyme Q10, etc. and whitening agent such as licorice, vitamin C, AHA, arbutin, etc.

However, there are some cosmetic actives that can easily be damaged by heat, light, etc. which have been focused on 2-ethylhexyl-p-methoxycinnamate (EHMC), ascorbyl palmitate and astaxanthin.

***Trans*-2-ethylhexyl-p-methoxycinnamate** (*trans*-EHMC):

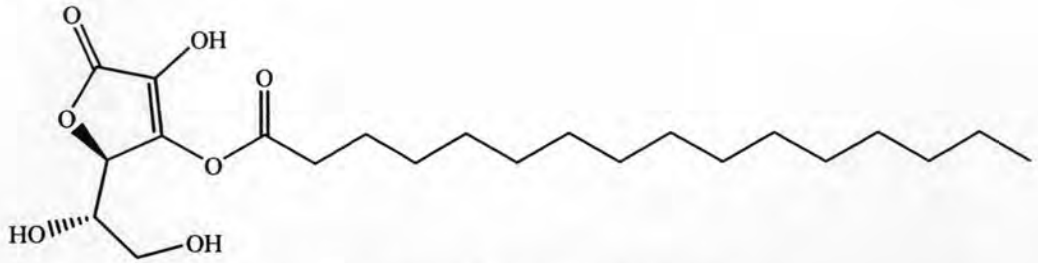



Structure of *trans*-EHMC

*Trans*-EHMC represents the most widely used sunscreen compound [63]. *Trans*-EHMC is classified as an UVB filter in accordance to its higher absorption in the shorter wavelength region (290-320 nm) of the solar UV radiation. Several studies have demonstrated that *trans*-EHMC is unstable following irradiation both in solution and in emulsion formulations [64].

In order to ensure adequate efficacy of this sunscreen agent, there is a need for new carrier system with enhanced *trans*-EHMC photostability. In this project, we have been focused on biocompatible nanoparticles delivery systems for the prevention of the isomerization of *trans*-EHMC.

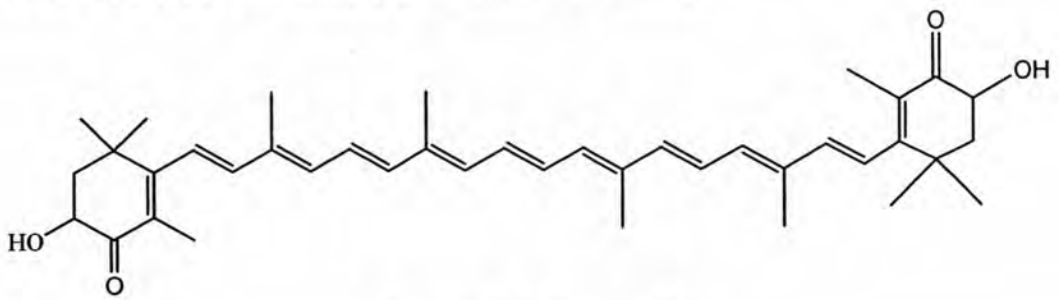
**Ascorbyl palmitate**:




Structure of ascorbyl palmitate

Ascorbyl palmitate is a derivative of L-ascorbic acid that is almost insoluble in water at room temperature. It has been used in cosmetic and dermatological products because the compound possesses many favourable effects on the skin. It is a strong antioxidant that can scavenge and destroy reactive oxidizing agents and free radicals, which are important in the processes of skin ageing [65]. It also improves the elasticity of skin and reduces wrinkles by stimulating collagen synthesis. However, the use of ascorbyl palmitate in cosmetic and pharmaceutical products is limited due to its low stability especially under aerobic conditions and light exposure [66].

**Astaxanthin**: (3, 3'-dihydroxy-β-β'-carotene-4-4'-dione):




Structure of astaxanthin

Astaxanthin is a natural color carotenoid found in salmon, shrimps, krill and crab, It is carotenoid pigment astaxanthin that has important applications in the nutraceutical, cosmetic, food and feed industries. Haematococcus pluvialis is the richest source of natural astaxanthin [67]. Astaxanthin is a strong colouring agent, a potent antioxidant, excellent prevention of lipid peroxidation and effective anti-inflammatory.

As most carotenoids, astaxanthin is highly unsaturated molecule and thus, can easily be degraded by light, thermal or oxidative processes during the manufacture and storage of foods. This can cause the loss of their nutritive and biological desirable

flavor or aroma compounds. Generally, carotenoids are found in nature as all-trans molecules in which all the double bonds are in the *trans* configuration [68]. It is also well known that high temperature and light conditions may promote the isomerization to the *cis* forms. The *cis* isomers of the provitamin A carotenoids have less activity than their corresponding all-*trans* carotenoids [69].

## 1.4 Literature Reviews

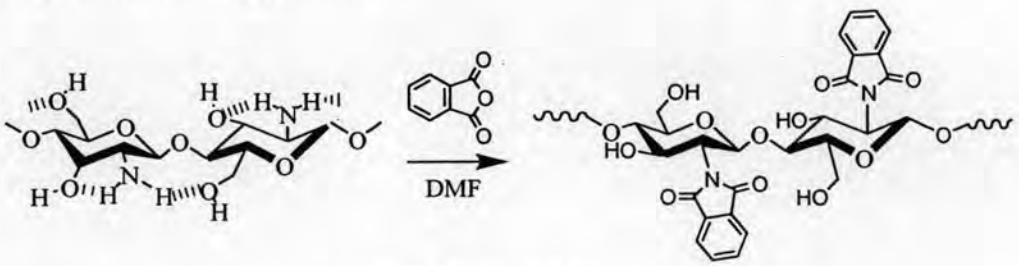
### Chitosan

*Derivatization of Chitosan*

The chemical structure of chitosan exhibits high crystallinity through inter- and intramolecular hydrogen bond network. Combining with the high molecular weight developed naturally, chitosan has poor solubility property. In order to overcome the poor solubility of chitosan in water or organic solvents, chemical modification of chitosan has been carried out.

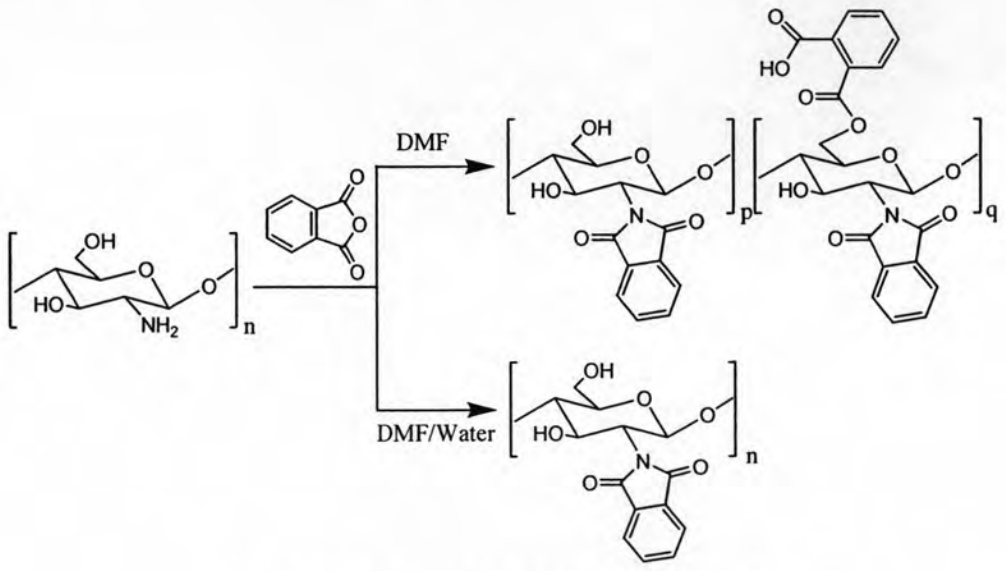
In 1980, Fujii *et al.* [70] demonstrated that the derivative achieved from the complete N,O-polyacylation of chitosan with an excess of long-chain acid chloride can be soluble in some organic solvents such as pyridine, dimethylsulfoxide and N,N'-dimethylacetamide.

In 1991, Nishimura *et al.* [71] 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-phthaloylchitosan 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 *N,N'*-dimethylformamide, dimethylsulfoxide and pyridine.



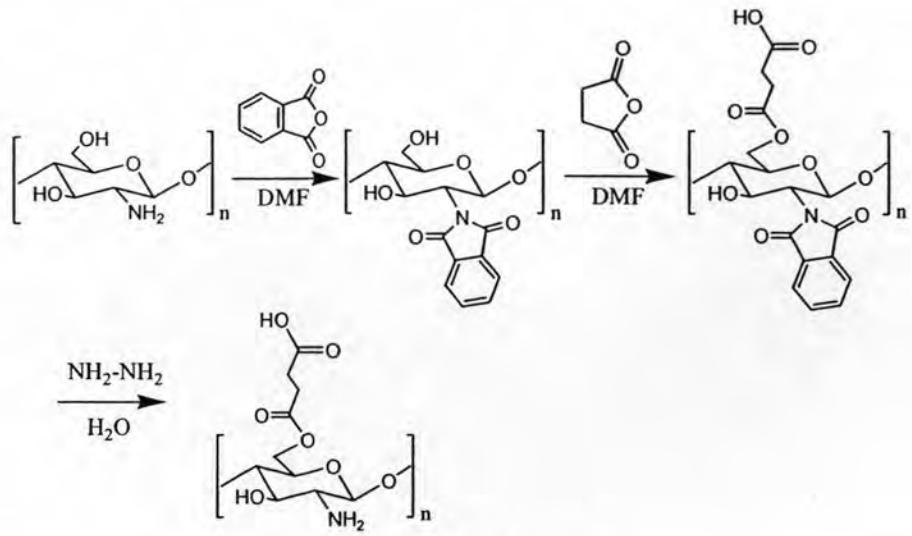

*N*-Phthaloylchitosan

In 2001, Kulita *et al.* [72] studied chemiselective N-phthaloylation of chitosan which could be accomplished successfully in one step using DMF containing 5% water as solvent. Crystallinity of N-phthaloylchitosan was observed. The N-phthaloylchitosan exhibited high affinity for organic solvents, although somewhat lower than that of N-phthaloylchitosan with additional O-phthaloyl groups.




N-phthaloylation / O-phthaloylation reaction

In 2003, Zhang *et al.* [73] 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 through the hydroxyl functionality marks the significance of this study. This 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.




O- Succinyl-chitosan

*Chitosan as a material for drug delivery system*

Owing to the unique properties of chitosan, i.e., biodegradability, biocompatibility, bioactivity and non-toxicity, chitosan is satisfied to use as drug carrier in pharamaceutical, medical and biotechnological fields.

In 1992, Ohya *et al.* [74] prepared 6-O-carboxymethyl chitin (CM-chitin) conjugated 5-fluorouracil (5FU) via pentamethylene and monomethylene spacer groups. The obtained CM-chitin/5FU drug showed the slow release of 5FU and exhibited emarkable antitumor activity against leukemia.

In 1992, Thanoo *et al.* [75] prepared the chitosan microspheres by emulsion cross-ling of chitosan solution in paraffin oil as an external medium with glutaraldehyde using dioctyl sulfosuccinate as the stabilizing agent. Addition of stabilizing agent during particle formation produced microspheres with spherical geometry and smooth surfaces. Encapsulation efficiencies up to 80% were achieved for theophylline, aspirin or griseofulvin. These microspheres were used to study the drug release rates, which were influenced by cross-link density, particle size and initial drug loading.

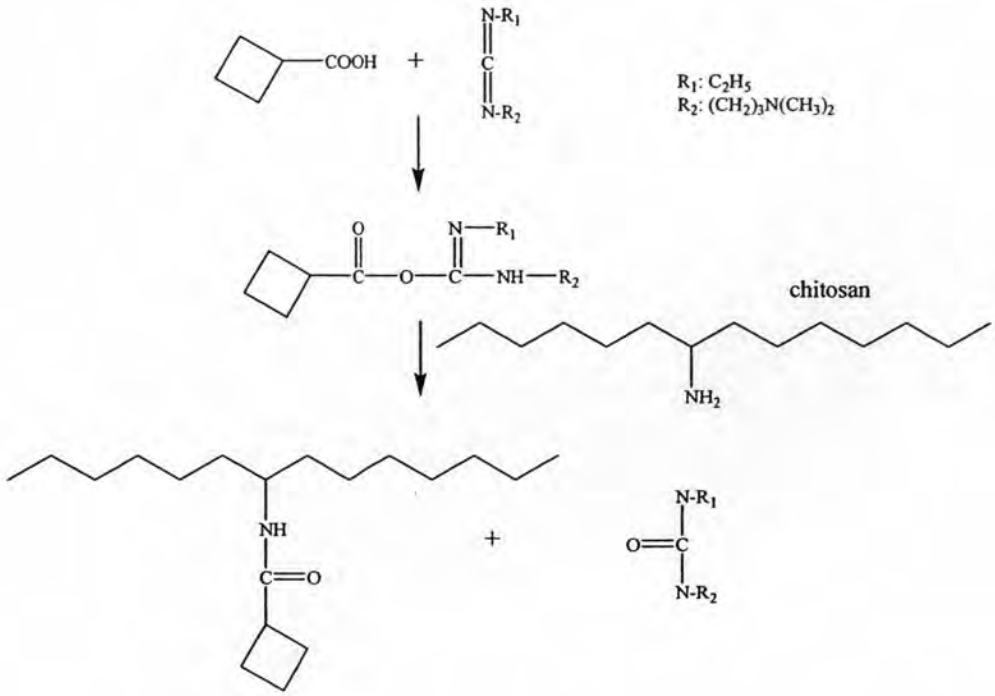
In 1998, Conti et al. [76] produced microparticles by exposing the spray-dried particles to vapors containing cross-linking agents. Cetylpyridinium chloride, an anti-infective agent, was incorporated into chitosan microspheres produced by spray-drying technique. Extent of cross-linking was controlled by the time of exposure to cross-linking agent.

In 2002, Pan et al. [77] prepared the insulin-loaded chitosan nanoparticles by ionotropic gelation of chitosan with tripolyphosphate (TPP) anions. The ability of chitosan nanoparticles to enhance the intestinal absorption of insulin and the relative pharmacological bioavailability of insulin was investigated by monitoring the plasma glucose level of alloxan-induced diabetic rats after the oral administration of various doses of insulin-loaded chitosan nanoparticles. The positively charged, stable chitosan nanoparticles showed particle size in the range of 250-400 nm. Insulin association was up to 80%.

In 2002, Mi et al. [78] prepared chemically modified porous chitosan beads by introducing of quaternary ammonium and aliphatic or aromatic acyl groups in order to interact with an anti-inflammatory drug, indomethacin, via the electrostatic interaction and hydrophobic interaction. The pore size and the porosity of beads were depending on the synthesis conditions, i.e., initial polymer concentration, pH value, and concentration of the casting agent (tripolyphosphate aqueous solution).

In 1997, Ouchi et al. [79] prepared water-soluble poly(ethylene glycol) grafted chitosan (PEG-g-chitosan) through the chemical modification of chitosan, and investigated its aggregation phenomenon in aqueous solution. PEG-g-chitosans having a degree of PEG of 25 and 55 mol % sugar unit$^{-1}$ were found to form aggregates due to intermolecular hydrogen bonds in aqueous solution. These PEG-g-chitosan aggregates could take up a small hydrophobic molecule such as PNA. PNA taken up into the aggregates could be released from the aggregates by changing the pH to an acidic condition. Therefore, this PEG-g-chitosan aggregate can be expected to be used as a pH dependent material such as a drug carrier for drug delivery systems.

In 1998, Lee et al. [80] designed a new carrier for DNA delivery by conjugating chitosan with deoxychoric acid (hydrophobic moiety). The amphiphilic macromolecule formed self-assemblies of self-aggregates to associate with DNA and transfer *in vitro*.




A scheme of the coupling mechanism between chitosan and deoxycholic acid using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) through amide linkage formation

In 1999, Ohya et al. [81] conjugated PEG to soluble chitosan via an amide linkage. The conjugated product formed self-aggregates at basic pH and could entrap insulin due to electrostatic interactions between the unconjugated chitosan and the anionic residues of the protein. The release rate of insulin was controlled by the degree of PEG substitution: the higher the PEG substitution, the more rapid release of insulin.

In 1999, Calvo *et al.* and Fernandez-Urrusuno *et al.* [82] modified the surface of chitosan nanoparticles by coating poly(ethylene glycol) to the surface of pre-formed nanoparticles via covalent amide bonds between the free amine group of nanoparticles and methoxyPEG. These chitosan nanoparticles showed the effective association and protein delivery such as bovine serum albumin (BSA), tetanus toxoid, diphtheria toxoid and the peptide insulin.

In 2003, Yoksan *et al.* [83] grafted poly(ethylene glycol) methyl ether terminated carboxylic acid (mPEG-COOH), hydrophilic chain, onto the hydroxyl group of N-phthaloylchitosan via homogeneous reaction. The grafted product formed self aggregates into nanoparticles. The product gives a milky solution when dispersed

in water and a series of solvents. The spheres obtained from mPEG with $M_n$ = 5000 Dalton show the average sizes about 80-100 nm.

*The development of sunscreens*

It is well known that UV radiation of the sun is harmful to the skin and of potential danger for the health. Therefore, it is very important to use sunscreen agents to protect oneself from the effects of UV rays. Health agencies world-wide also recommend the use of sunscreens as a means of lowering the risk of developing skin cancer.

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 octyl-methoxycinnamate (EHMC), which possesses a high molar absorption coefficient (ε), approximately 22000-24000 $M^{-1}cm^{-1}$ and shows only few allergic reactions to human skin. Nevertheless, many reports have shown that amounts of EHMC can be penetrated into human body and possible toxicity and phototoxicity of EHMC was suspected. Moreover, dibenzoylmethanes and benzophenones which is popular UVA filter were also found in human body. Many researchers have indicated trasdermal penetration of many other small molecule UV filters.

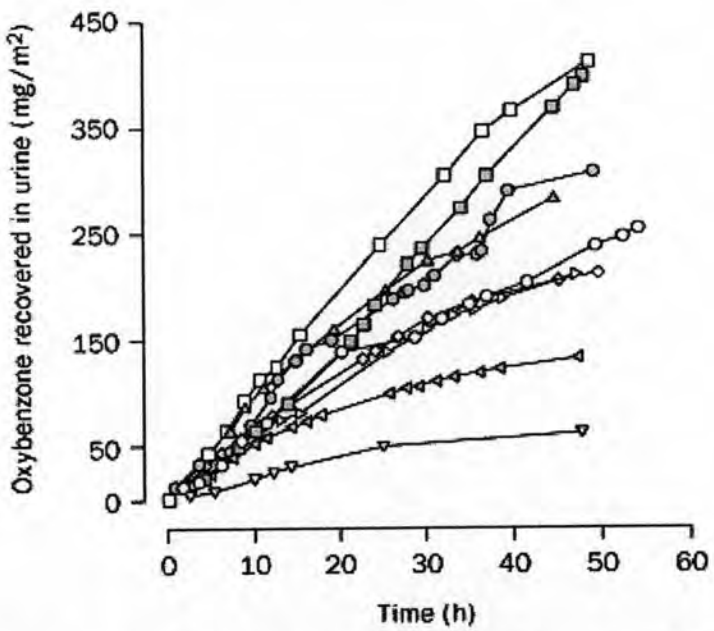
In 1995, J. Hany and R. Nagel [84] detected benzophenone-3 and EHMC in human breast milk and in the same year, U.H. Leweke and B.C. Lippold1 studied transdermal penetration through human skin layer of various sunscreens. The study presented amount absorption range between 10-89 mg of octyl dimethyl p-aminobenzoic acid, 4-isopropyl-dibenzoylmethane, 3-(4-methylbenzylidene)-camphor, isoamyl-p-methoxy-cinnamate and oxybenzene after treating the whole skin surface (1.8 $m^2$) with a saturated solution for one hour.

In 1996, P. Treffel and B. Gabard [85] found that the amounts of B3, EHMC, and octylsalicylate recovered from tape-stripped stratum corneum suggest that these UV filters penetrate into the epidermis.

In 1996, Lazar et al. [86] used several types of emulsions containing EHMC and butylmethoxy dibenzoylmethane for their *in vitro* percutaneous absorption study. They created a diffusion cell with the stratum corneum in contact with the sunscreen

solution and the dermis in contact with the receptor fluid (BSA, NaCl and ether). The receptor fluids were analyzed using detection at 325 nm and they were able to evaluate the amount of UV filters present in the various samples. The degree that the chemical filters penetrate into the skin is dependent on the type of emulsion and the vehicle properties. In fact, Lazar et al. found oily external phases are the best sunscreen preparations, since they have low penetration rates for the UV filters and stay on the stratum corneum rather than diffusing through the epidermis.

In 1997, C.G.J. Hayden [87] found that oxybenzone, a benzophenone derivative commonly used throughout the world to make sun-products with especially high sun protection factors (SPF), can penetrate through the stratum corneum and be absorped in human urine, with up to 1–2% of the applied amount into the body. Moreover, B3 has also been detected in breast milk.

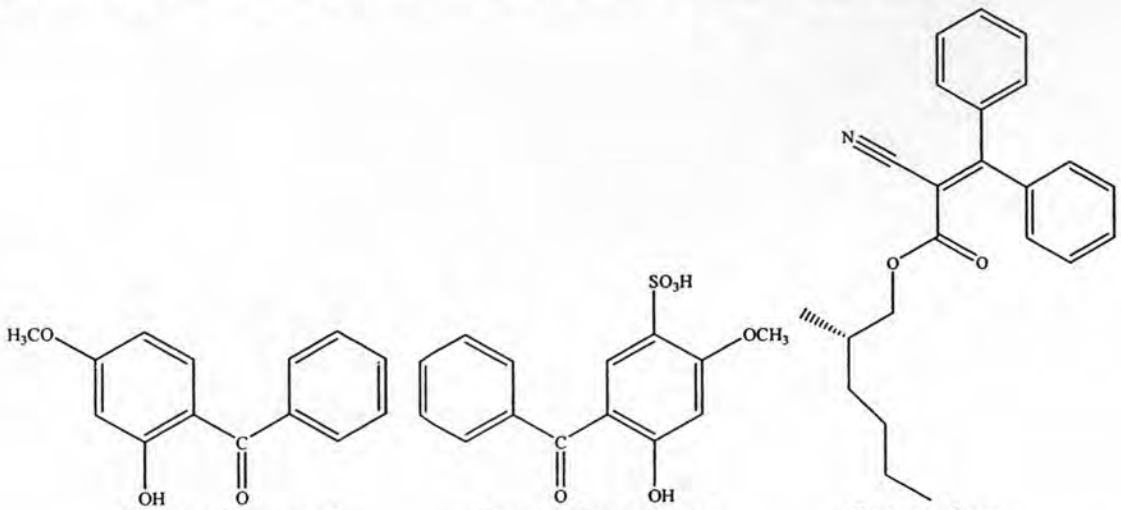



Oxybenzone recovered in urine as unchanged oxybenzone and metabolites after topical application of commercially available SPF 15+ sunscreen to nine human volunteers

In 1997, Heyden et al. [88] reported that EHMC could penetrate through the skin layers into blood circulation. In this study, EHMC could be recovered from milk of human volunteers.

In 1999, G. Potard [89] studied *in vitro* percutaneous penetration of EHMC, benzophenone-3, benzophenone-4, octyl triazone, and octocrylene in various skin

layers by stripping technique with rapid HPLC for the quantification. The result obtained after and exposure time of 16 hours on human fresh skin indicated that UV filters could penetrate into stratum corneum, epidermis and dermis. In addition, their mean amount found in the receptor fluid is zero or very low, except for benzophenone-3. Potard et al. also mentioned that the skin of different people reacts differently and, therefore, penetration varies among individuals as well.



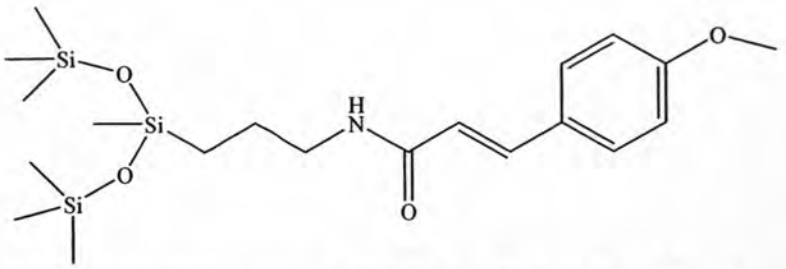

Benzophenone-3 Benzophenone-4 Octocrylene

In 1999, V.K. Gupta [90] studied penetration of EHMC and benzophenone-3 through micro-Yucatan pig skin by diffusion cell technique. They observed that the two sunscreens reached the stratum corneum within an hour and found that benzophenone-3 penetrated skin to a greater extent than EHMC and the amounts penetrated into viable skin and receptor fluid increased slowly over time.

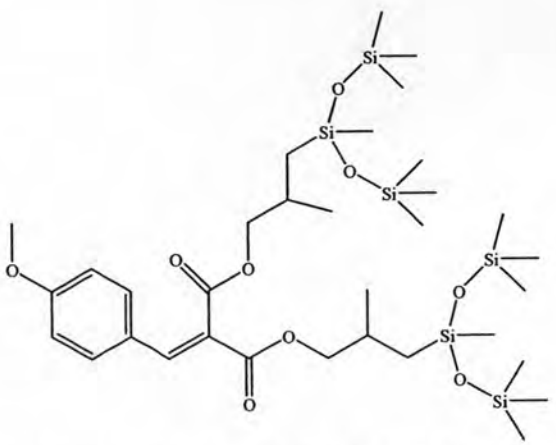
In 2000, Benech-Kieffer et al. [91] studied the percutaneous absorption of EHMC sunscreens in vitro. EHMC absorptions were detected in both pig and human. The correlation of qualitative data between pig skin and human skin was very good. This reseach confirmed the EHMC absorption through both pig's and human's skin.

Existing solutions for transdermal absorption of sunscreen included encapsulation of sunscreens into various carriers, making UV filter as polymeric molecules and adjusting formulation to reduce the penetration rate.

In the U.S. Pat. No. 6080880 (2000), Richard et al. [92] prepared novel cinnamamide, benzalmalonamide and benzalmalonate compounds (UV absorptive chromophores) bearing short-chain silicone substituents on the aromatic moieties. The compounds possess, besides very good solubility in fatty materials, improved cosmetic properties.



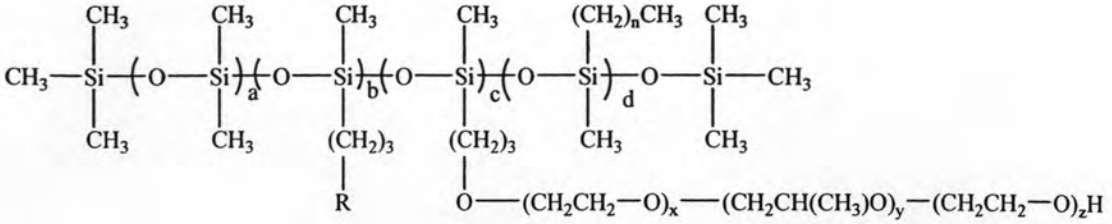
4-Methoxy-N-[3-[1, 3, 3, 3-tetramethyl-1-[(trimethylsilyl)oxy]disiloxanyl]propyl] cinnamamide



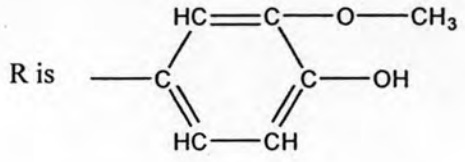
Di-[2-methyl-3-[1,3,3,3-tetramethyl-1-[(trimethylsilyl)oxy]-disiloxanyl]propyl]4-methoxybenzalmalonate

In 2001, Godwin et al. [93] determined the influence of ethoxydiglycol (Transcutol ® CG's) concentration on the transdermal permeation and skin accumulation of two ultraviolet (UV) absorber, 2-hydroxy-44-methoxybenzophenone (oxybenzone) and 2-octyl-4-methoxicinnamate. The results demonstrated that the inclusion of Trancutol ® CG in sunscreen formulaltions increased the skin accumulation of the UV absorbers oxybenzone and cinnamate without a concomitant increase in trasdermal permeation.

U.S. Pat. No. 6346595 (2002) by O'Lenick, Jr. [94] disclosed novel silicone compounds that contain a UV-absorber and a polar alkoxylated group. The presence of the polar alkoxylated group not only has a dramatic effect upon solubility of the sunscreen, but also shifts the UV absorption properties, making it possible to synthesize products that have a specified UV absorption property.

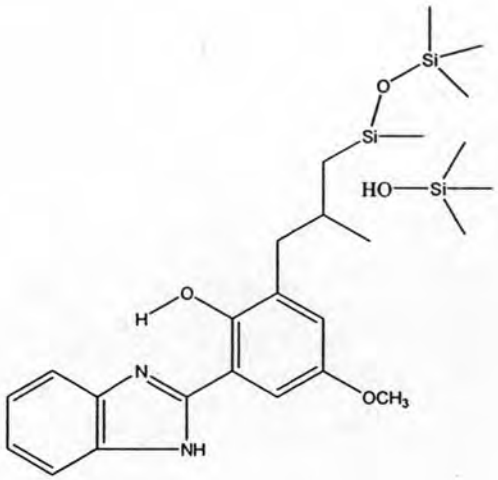


Structure of dimethicone copolyol polymer



a is an interger ranging from 0 to 2000;
b is an interger ranging from 1 to 20;
c is an interger ranging from 1 to 20;
d is an interger ranging from 10 to 20;
n is an interger ranging from 0 to 20;
w is an interger ranging from 0 to 20;
x is an interger ranging from 0 to 20;
y is an interger ranging from 0 to 20;
z is an interger ranging from 0 to 20.

U.S. Pat. No. 6376679 (2002) [95] disclosed the grafting of one or more benz-x-azole groups to a silicone chain. New compounds with excellent filtering properties in UV-A and/ or UV-B radiation range, very good solubility in the commonly used organic solvents and particularly fatty substances such as oils, could be obtained. They could be used as solar radiation filters in, or for the preparation of, cosmetic compositions intended for the protection of the skin and/or hair against ultraviolet radiation.



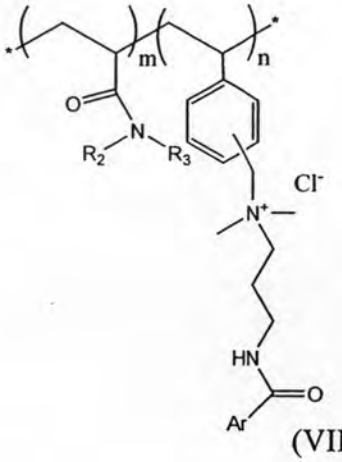

Structure of the 2-[5-methoxy-2-(2-methyl-allyloxy)-phenyl]-1H-benzimidazole

In 2003, Hongchinakorn [96] grafted 4-methoxy cinnamic acid on silicone. The obtained product, gave UV absorption profile similar to that of EHMC but with much lower skin permeation and better photostability property.

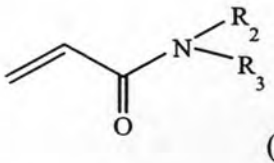
In 2003, Jimenez et al. [97] reported the evaluation of the *in vitro* trasdermal permeation and skin accumulation of EHMC through pig skin. They determined the quantity of EHMC in different pig skin layers (stratum corneum, viable epidermis, dermis and receptor fluid). The study showed that by encapsulating EHMC into nanocapsule, skin permeation of EHMC was decreased.

U.S. Pat. No. 7087692 (2006) by Koshti et al. [98] disclosed the synthesis the water soluble polymers containing cinnamidoalkylamines and/or benzamidoalkylamines. The polymers contain cationic centres for enhanced substantivity. When these water-soluble polymers are applied to skin, the temperature of human body and the salt content of water make them insoluble and hence do not get easily washed off either by sweat or sea water. These properties make these macromolecules useful for personal care as well as fabric care products.

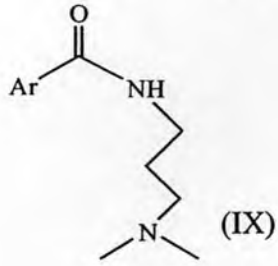
The synthesis of the polymer VII was carried out in three steps, (1) synthesis of cinnamidoalkylamines and/or benzamidoalkylamines (VIII), (2) copolymerization of monomer IX and vinyl benzyl chloride and (3) functionalisation of the obtained copolymer by quaternisation using VIII.



(VII)



(VIII)



(IX)

Wherein:

ArCo is an UV-absorbing moiety of an organic sunscreen acid or mixtures of organic sunscreen acids selected from *p*-methoxy cinnamic acid and *p*-dimethyl amino benzoic acid;

$R_2$ and $R_3$ are selected from hydrogen, alkyl and cycloalkyl group containing from 1 to 6 carbon atoms;

m is an integer from 5 to 9;

n is an integer between 1 to 5;

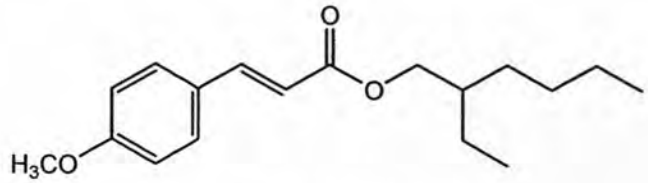
m+n is equal to 10

*Cosmetic active*

For many years, several efforts have been made to solve stability, colour and various other problems of cosmetic actives. One interesting way is encapsulation into micro- or nanoparticles.

*2-Ethylhexyl-p-methoxycinnamate (EHMC)*

In 2002, Perugini et al. [99] investigated the influence of nanoparticle-based systems on the light-induced decomposition of the sunscreen agent, *trans*-2-ethylhexyl-p-methoxycinnamate (*trans*-EHMC). They illustrated that the photodegradation of the sunscreen agent in emulsion vesicles was reduced by encapsulation the sunscreen into the poly-D,L-lactide-co-glycolide (PLGA) nanoparticles. They also indicated that encapsulation with ethylcellulose (EC) nanoparticles gave no significant effect on EHMC's photostability.



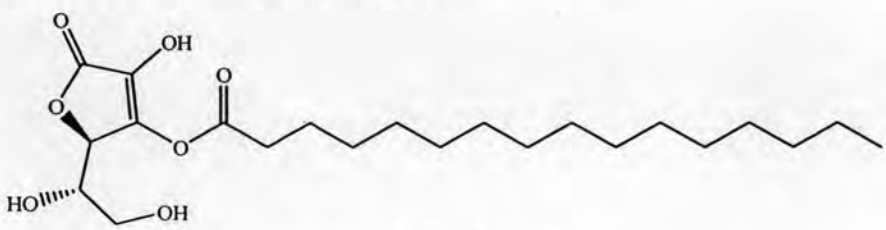

*Trans*-EHMC

In 2003, Yener *et al.* [100] prepared solid lipid microspheres (SLM) as carriers for EHMC in order to decrease the release and penetration rate of this UV absorber. They demonstrated that incorporation of EHMC into SLM also enhanced the photostability of EHMC while the effectiveness in UV protection of EHMC in liposphere form was nearly the same as that of the free form.

*Ascorbyl palmitate*

In 2001, Spiclin et al. [101] studied the stability of both ascorbyl palmitate and sodium ascorbyl phosphate in microemulsions (oil in water and water in oil) for topical use as carrier systems and found that the hydrophilic sodium ascorbyl phosphate is more stable than the lipophilic ascorbyl palmitate. He demonstrated that the stability of ascorbyl palmitate is highly dependent on its initial concentration, its location in the microemulsion, the amount of oxygen dissolved in the system and the storage conditions.

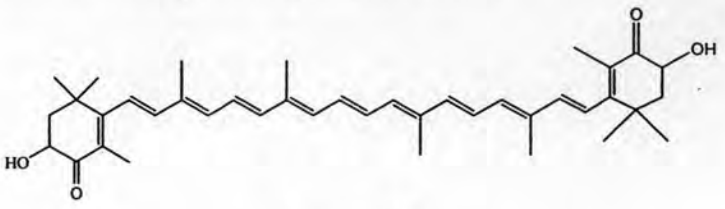
In 2003, Julijana *et al.* [102] illustrated that the long-term stability of the model drug ascorbyl palmitate (AP) depend on the composition of, and drug distribution in the colloidal carrier systems. In particular, the drug protecting ability depends on the location of unstable part of the active compound in the carrier system. In AP, where the hydrophilic moiety is reactive to oxidation, the molecule is most stable in systems in which this part is least exposed to the hydrophilic environment. Therefore, AP was more stable in solid lipid nanoparticle (SLN) and non-hydrogenated soybean lecithin (NSL) liposomes than in hydrogenated soybean lecithin (HSL) liposomes and microemulsions.




Ascorbyl palmitate

*Astaxanthin*

In 2002, Lorenze [103] 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 delivery by a cream in a therapeutically effective dose.




Astaxanthin

However, astaxanthin is a highly unsaturated molecule and thus, can easily be damaged by heat or light, which can cause the loss of biological properties.

In 2003, Higuera-Ciapara et al. [104] studied the stability of astaxanthin in microcapsules under storage at 25, 35 and 45°C for 8 weeks by measuring isomerization and loss of concentration of astaxanthin. Results showed that the microencapsulated pigment did not suffer isomerization nor chemical degradation under the investigated storage conditions.

In 2006, Xiaolin Chen et al. [105] illustrated that the stability of the inclusion complex of astaxanthin with β-cyclodextrin against temperature and light was greatly enhanced compared to that of free astaxanthin.

## 1.5 Research goal

The objectives of this research can be summarized as follows:

1. To graft mPEG-COOH (and 4-methoxycinnamic acid) onto *N*-phthaloylchitosan.
2. To synthesize nanoparticle from chitosan derivatives (mPEG-4-methoxycinnamoyl-phthaloylchitosan- and mPEG-phthaloylchitosan).
3. To encapsulate the EHMC, ascorbyl palmitate and astaxanthin into mPEG-phthaloylchitosan nanoparticles.
4. To encapsulate the ascorbyl palmitate and astaxanthin into mPEG-4-methoxycinnamoyl-phthaloylchitosan nanoparticles.
5. To investigate photostability of 2-ethylhexyl-p-methoxycinnamate in mPEG-phthaloylchitosan nanoparticles.