CHAPTER I

INTRODUCTION

1.1 UV radiation

Ultraviolet is a term used to describe the wavelength between the blue violet range of the visible spectrum and the shorter wavelength or X-rays. The UV radiation component of sunlight is small but biologically important, consisting of the wavelengths between 100 and 400 nm. These are then further subdivided into four major bands; vacuum UV, UVC, UVB and UVA [1] (Figure 1.1.).

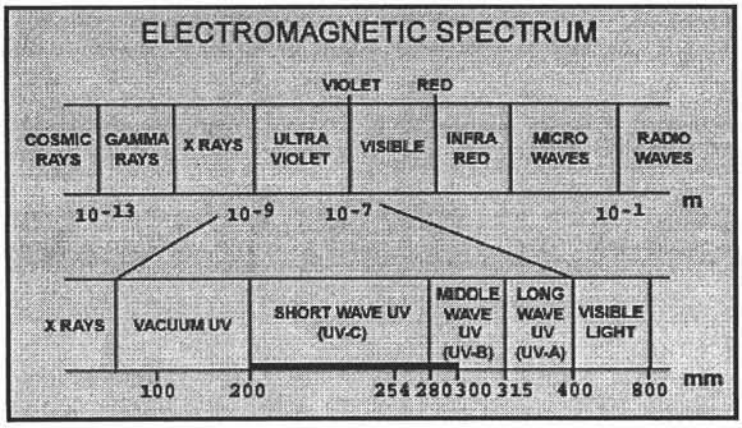


Figure 1.1 Electromagnetic spectrum [1].

The vacuum UV is a radiation with a wavelength less than 200 nm. Neither vacuum UV (100-200 nm) nor UVC (200-280 nm) reach the surface of the earth because of the absorption by ozone layer. On the other hand, the UVB (280-320 nm) and UVA (320-400 nm) can penetrate the ozone layer. The UVB radiation promotes vitamin D synthesis and increases skin pigmentation or tanning [2]. At the same time, UVB triggers various serious skin damages depending on light intensity and skin sensitivity. UVB inhibits or interferes DNA, RNA and protein synthesis [3], induces early and prolonged erythema responses that would lead to photo aging and skin cancer [3]. The UVB effects are direct in nature and do not require intermediate photo sensitizer because nucleic acids, proteins and many biological molecules can directly absorb UVB radiation [4].

The UVA can produce a significant amount of photo induced biological effects. The UVA indirectly damages cells through the reactive oxygen species [4-6]. UVA ray also influences a direct pigmentation and increases the biological effect from the UVB [2]. They induce both an immediate erythema response which diminishes within 2 hours and a delayed erythema response which reach a peak at 6 hours. This is opposite to the UVB induced delayed erythema which tends to reach a peak in 12 to 24 hours [3]. Although UVA is less energetic than UVB, it can, however, penetrate deeper into the dermis (Figure 1.2), while UVB can penetrate only into the stratum corneum and the epidermis. Both UVA and UVB contribute together to make photo aging and skin cancers.

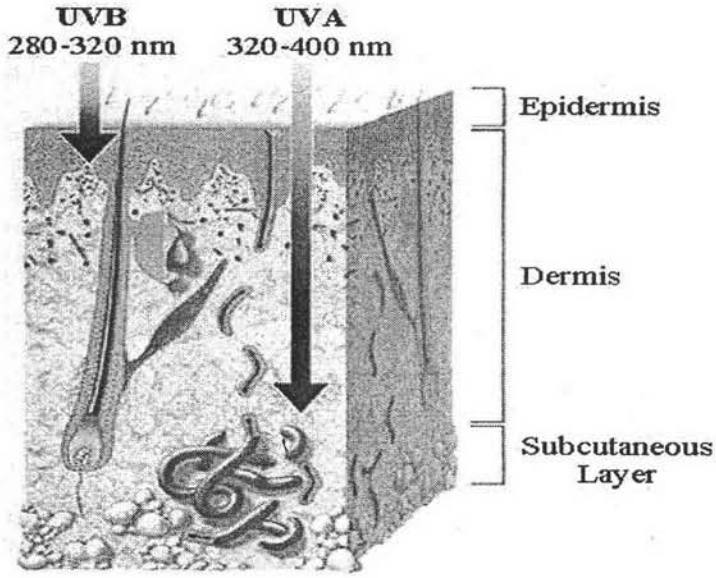


Figure 1.2 Penetrations of UVA and UVB radiations into the skin [3].

1.2 Sunscreen

As mentioned above, various damaging effects from UVA and UVB have been well realized. As a result, the use of sunscreen has become a common mean to prevent the damages from these radiations.

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

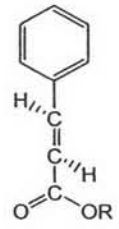
1.2.1 Physical blockers

A physical blocker is a chemical that reflects or scatters the UV radiation. Examples of physical blockers include zinc oxide, titanium dioxide, and red petrolatum. Physical blockers, if presented of sufficient quantities, will reflect all the UV radiation. They are currently being used in conjunction with chemical absorbers to achieve high sun protection factors (SPF).

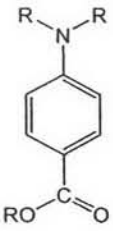
There are, however, still many debates on the safety of physical blockers. Titanium dioxide (TiO_2) has been reported to produce hydroxyl radicals under UVA irradiation and, therefore, can induce cytotoxicity [7, 8]. Since TiO_2 exhibits semiconductor properties, they have been used as photo catalyst for the degradation of organic pollutants in waste waters [7]. Some investigators have shown that photo excited titanium dioxide can cause cell death both *in vitro* and *in vivo* [9]. Penetrations of ultrafine TiO_2 particles have also been reported [10].

1.2.2 Chemical absorbers

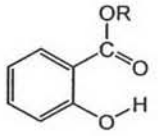
Chemical absorbers are organic molecules whose absorption bands are in UV region. Thus, it cloud help on absorption of harmful UV radiation. UVA absorbers are chemicals that absorb radiation in the 320-360 nm regions of the UV spectrum. Examples of UVA absorbers include benzophenone, anthranilate, and dibenzoyl methane (see Figure 1.3). UVB absorbers are chemicals that absorb radiation in the 280-320 nm regions of the UV spectrum. Examples of UVB absorbers include *p*-aminobenzoate (PABA) derivatives, salicylate, cinnamate and camphor derivatives (see Figure 1.3).



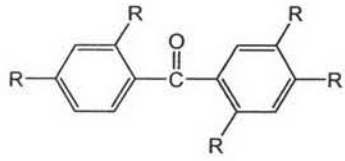
A. Cinnamate derivatives



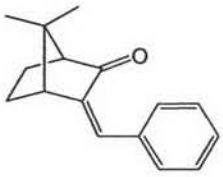
B. *p*-Aminobenzoate (PABA) derivatives



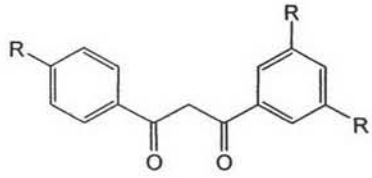
C. Salicylate



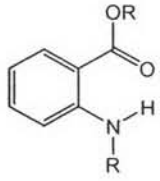
D. Benzophenone



E. Camphor derivatives



F. Dibenzoylmethane



G. Anthranilate

Figure 1.3 The sunscreen absorbers used in the sunscreen industry.

1.3 Mechanism of Sunscreen Action

Sunscreen chemicals are generally aromatic compounds conjugated with a carbonyl group. In many examples, 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 (280-390 nm) and convert to the remaining energy into longer wave (lower energy) radiation (usually above 700 nm). The process of sunscreen action is called "photophysical process", not a photochemical process and ideally no change in molecular structure should be observed. Mechanism of sunscreen action (Figure 1.4) 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.

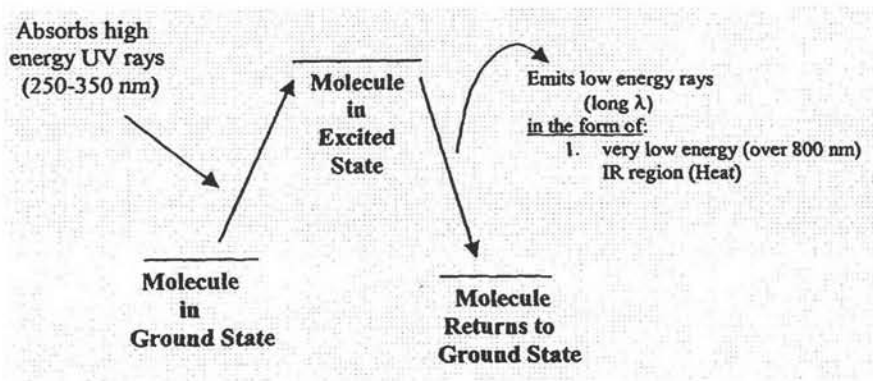


Figure 1.4 Mechanism of sunscreen action [3].

1.4 Factors affecting the UV Absorbance of Sunscreens

1.4.1 Effect of pH

The UV absorption spectra of acidic and basic compounds are affected by pH. In the case of acidic compounds, the use of alkaline conditions (pH>9) will result in the formation of anions that tend to increase delocalization of electrons. This electron delocalization decreases the energy required for the electronic transition in the UV spectrum, and hence a bathochromic shift is observed (longer wavelength). On the other hand, acidic conditions (pH<4) will result in the formation of cations with aromatic amines. A hypsochromic

shift (toward lower wavelength) occurs since the protonation of the unbounded lone pair electrons decreases resonance delocalization of the electrons.

1.4.2 Effect of Solvent

The use of different solvents in cosmetic formulations may profoundly influence the effectiveness of a sunscreen chemical. If the sunscreen is polar, then interactions with polar solvents will be quite extensive. This interaction with solvent stabilizes the ground state, thereby inhibiting electron delocalization. The net result would be a hypsochromic shift to lower wavelength. In contrast, if the sunscreen is less polar, then interactions with polar solvents would be stronger at the excited state, which is more polar than the ground state. This then lowers the energy requirements for the electronic transition; hence, a higher maximum wavelength (λ_{max}) would be expected, and bathochromic shift occurs.

1.4.3 Effect on the Extinction Coefficient

The effectiveness of a sunscreen chemical is assessed through the value of the extinction coefficient. Chemicals with high extinction coefficient values are more efficient in absorbing the energy of the harmful UV radiation than those with lower extinction coefficient values. Electronic transitions for any compounds may be characterized as symmetry allowed or forbidden. Symmetry allowed transitions generally have high extinction coefficients while symmetry forbidden transitions have lower extinction coefficients. The degree of resonance delocalization in a molecule can be used to predict the relative λ_{max}. A similar qualitative prediction for extinction coefficient is also possible. Usually the extinction coefficient values are reported in terms of "Molar Extinction Coefficient" or sometimes called "Molar Absorption Coefficient" or ε. The ε value is obtained experimentally using Beer's law:

$$A = \varepsilon bc$$

where A is absorbance

b is the cell path length (1 cm)

c is the concentration of the absorbing species in mol per liter

1.5 Curcumin

Curcumin can be extracted from turmeric (*Curcuma Longa* Linn) (Figture 1.5), a perennial herb, member of the Zingiberaceae (ginger) family. The plant grows to a height of three to five feet, and is cultivated extensively in Asia, India, China, and other countries with a tropical climate. It has oblong, pointed leaves and bears funnel-shaped yellow flowers. The rhizome is the portion of the plant used medicinally; it is usually boiled, cleaned, and dried, yielding a yellow powder. Dried Curcuma longa is the source of the spice turmeric, the ingredient that gives curry powder its characteristic yellow color. Turmeric is used extensively in foods for both its flavor and color. Turmeric has a long tradition of use in the Chinese and Ayurvedic systems of medicine, particularly as an anti-inflammatory agent, and for the treatment of flatulence, jaundice, menstrual difficulties, hematuria, hemorrhage, and colic. Turmeric can also be applied topically in poultices to relieve pain and inflammation. Two current researches are focused on turmeric's antioxidant, hepatoprotective, anti-inflammatory, anticarcinogenic, and antimicrobial properties, in addition to its use in cardiovascular disease and gastrointestinal.

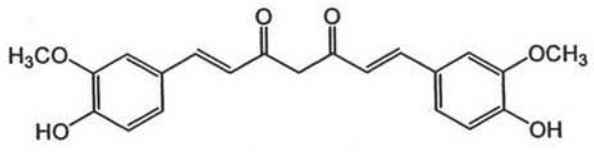
Figure 1.5 Turmeric or *Curcuma longa L.* (*Zingiberaceae*) [12].

1.6 Literature reviews

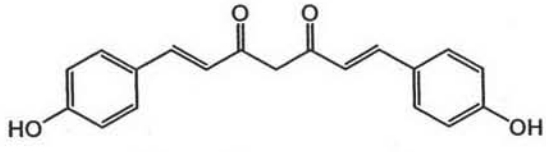
1.6.1 Curcumin

The coloring principle of turmeric was isolated in the 19th century and was named curcumin. Curcuminoids refer to a group of phenolic compounds present in turmeric, which are chemically related to its principal ingredient curcumin. Three curcuminoids were isolated from turmeric e.g., curcumin, demethoxycurcumin and bisdemethoxycurcumin [11] (Figure 1.6). All three impart the hallmark yellow pigmentation to the *C. longa* plant and particularly to its rhizomes. Although the chemical structure of curcumin was determined in the 1973 by Roughley and Whiting [12], which the major constituent curcumin (1,7-bis[4-hydroxy-3-methoxyphenyl]-1,6-heptadiene-3,5-dione) is in the most important fraction of C. *longa* L. (Figure1.5). Recently the potential uses of curcuminoids in medicine have been studied extensively. The structure of curcumin as diferuloylmethane was confirmed by the degradative work.

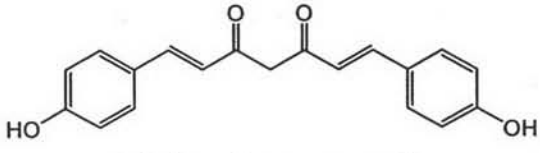
In 1995, Majeed and coworkers [13] reported that boiling with alkali; curcumin gave vanillic acid and ferulic acids whose structures were established. Fusion with alkali yielded protocatechuic acid and oxidation with potassium permanganate yielded vanillin. On hydrogenation, mixtures of hexahydro- and tetrahydro-derivative were obtained. Based on these, the structure of curcumin was established as diferuloylmethane.



Curcumin



Demethoxycurcumin



Bisdemethoxycurcumin

Figure 1.6 Structures of curcumin, demethoxycurcumin and bisdemethoxycurcumin.

1.6.2 Biological activities of curcumin

In 1973, Srimal and coworkers [14] reported the pharmacological action of curcumin e.g. the compound was effective in acute as well as chronic models of inflammation. The potency of this drug is approximately equal to phenylbutazone in the carrageenin-induced edema test, but it is only half as active in the chronic experiments. It was observed that curcumin was less toxic than the reference drug (no mortality up to a dose of 2 g/kg).

In 1994, Pulla and coworkers [15] reported that curcumin is a good antioxidant and inhibits lipid peroxidation in rat liver microsomes, erythrocyte membranes and brain homogenates.

In 1999, Ahsan and coworkers [16] studied structure activity relationship between curcumin, bisdemethoxycurcumin and demethoxy curcumin. Curcumin was found to be most effective in the DNA cleavage reaction, rate of formation of hydroxyl radicals and a reducer of Cu (II) followed by bisdemethoxycurcumin and demethoxycurcumin.

In 2001, Kapoor and coworkers [17] reported that curcumin could be a powerful antioxidant to repair both oxidative and reductive damage caused to proteins by radiation.

In 1999, Araujo and coworkers [18] reported the anti-protozoal activity of curcumin and some semi-synthetic derivatives against tripanosomatids in promastigotes (extracellular) and amastigotes (intracellular) forms of Leishmania amazonensis. It was reported that curcumin has an excellent activity (LD_{50} = 24 mM or 9 mg/ml) and the semi-synthetic derivative, methylcurcumin (a non-phenolic curcuminoid), has the best action with a LD_{50} < 5 mg/ml and LD_{90} = 35 mM against promastigotes forms. This derivative was tested in vivo in mice and showed good activity with 65.5% of inhibition of the lesion size of the footpad of the animals, when compared with the group inoculated with the parasites alone. Another interesting point mentioned is that they did not observe any inflammatory reaction in the area where the drugs were injected, perhaps because curcuminoids are potent inhibitors of inflammation.

In 1993, Sui and coworkers [19] reported inhibition of HIV-1 and HIV-2 proteases by curcumin and curcumin boron complexes. Simple modification of the curcumin structure rise the IC_{50} value complexes of the central dihydroxyl groups of curcumin with boron lower the IC_{50} to a value as low as 6 and 55 mm, respectively, for HIV-1 and HIV-2, whereas curcumin showed 100 and 250 mm.

In 1995, Mazumder and coworkers [20] demonstrated that curcumin has an antiviral activity, being a HIV-1 integrase inhibitor (IC_{50}= 40 mM) and suggested that curcumin analogs could be developed as anti-AID's drugs. Data showed that curcumin inhibited the replication of HIV-1 integrase protein.

In a recent review on cancer chemoprevention by dietary constituents, it was mentioned that curcumin has tumoursuppressing properties in rodent models of carcinogenesis, and interfere with cellular processes involved in tumour promotion and progression (Gescher, Sharma, and Steward, 2001) [21]. Antitumour promoting potential of curcumin has also been recently reviewed (Surh, 2002) [22].

In 2002, Usha and coworkers [23] reported turmeric and its coloring principle, curcumin, inhibit the formation of mutagens and also inhibited the mutagenicity of pyrolysates with or without metabolic activation.

In 2000, Rajakrishnan and coworkers [24] reported that curcumin can also protect against inflammation-related changes in the liver prostanoids in an animal model of alcohol-caused hepatic injury linked to increased activity in serum enzymes aspartate transaminase and alkaline phosphatase. When the diet of the ethanol-consuming rats was supplemented with curcumin, not only the activity of these serum enzymes was decreased but there was also a reduction in the abnormally raised levels of prostaglandins E_1 and E_2 in liver as well as in kidney and brain.

In 2000, Chuang and coworkers [25] have shown that gavages administration of 200 mg of curcumin suppresses diethyl nitrosamine-induced inflammation and hyperplasia in rats, as shown by histopathological examination.

In 2003, Christos and coworkers [26] preferred composition containing skin whitening blend containing bearberry and antioxidant, such as tetrahydrocurcumin, is provided. The skin whitening blend may also include a sunscreen component. Similarly in same year, Muhammed Majeed and coworkers [27] reported tetrahydrocurcumin can be protected skin against from free radicals and UVB rays.

In 2005, Yoshiyuki Mizushina and coworkers [28] found that monoacetyl curcumin ([1E,4Z,6E]-7-(400-acetoxy-300-methoxyphenyl)-5-hydroxy-1-(40-hydro xy-30-methoxyphenyl)hepta-1,4,6-trien-3-on), a new inhibitor of eukaryotic DNA polymerase λ , was a stronger pol λ inhibitor than curcumin, achieving 50% inhibition at a concentration of 3.9 μM.

In 2006, Li Lin and coworkers [29] synthesized 4-Ethoxycarbonylethyl curcumin (ECECur) which is a current drug candidate for the treatment of prostate cancer. Due to problems inherent in the tautomerism of ECECur, 4-fluoro-4-ethoxycarbonylethyl curcumin and 4-ethoxycarbonylethylenyl curcumin were designed and synthesized which were intermediated for inhibitory activity against androgen receptor transcription in LNCaP and PC-3 prostate cancer cell lines.

1.6.3 Sunscreen

Sunscreen products are routinely tested for their ability to delay the onset of erythema (sunburn) in skin exposed to UV radiation. Regulatory requirements for the approval of sunscreen agents are varied. In the USA, sunscreens are regarded as drugs whereas in Europe, they are classified as cosmetics. The majority of the commercially available sunscreens today are a combination of agents from several chemical agents. Para-amino benzoic acid (PABA) was an early chemical sunscreen agent that was frequently associated with contact and photo contact sensitivity reactions, had poor substantivity characteristics, and often discolored clothing and toxic [4, 5]. This compound is rarely found in sunscreen formulation today. Other sunscreen agents include compounds from the salicylate, cinnamate, benzophenone, anthranilate, and dibenzoylmethane groups. Among them, cinnamate derivatives are most

widely used sunscreen compounds in many countries. Many research groups have demonstrated that octyl methoxycinnamate (OMC) is quite safe concerning allergic contact (AC) and photoallergic (PA) effects of the compound [30, 31]. This makes OMC now is the most widely used UVB filter in cosmetic formulation worldwide.

Many reports have shown that substantial amounts of applied cinnamate derivatives and other sunscreens can be absorbed into human's skin layers [22-25]. For examples, in 1995 Hany and coworkers [36] reported that benzophenone-3 (BZ-3) and OMC were absorbed through the skin layers. In this study, OMC could be recovered from milk of human volunteers. Similarly in 1997 Hayden and coworkers [33] also reported that oxybenzone (benzophenone derivative) was absorbed through the skin layers. In this study, oxybenzone could be recovered from urine of human volunteers.

In 2002, Gonzalez and coworkers [37] reported that benzophenone-3 (BZ-3) was absorbed through the skin layers. In this study, BZ-3 could be recovered from urine, faces and blood of human volunteers.

In 2003, Chateiain and coworkers [38] studied penetration of UV filters (benzophenone-3 (BZ-3), ethylhexyl methoxycinnamate, butyl methoxydibenzoyl methane, ethylhexyl salicylate and homosalate) which found that ethylhexyl methoxycinnamate and BZ-3 could be recovered in the stratum corneum of the human skin (Franz Cells).

1.6.4 Catalyst for esterification

Common catalyst used for esterification such as acid chloride, p-toluene sulfonic acid, ect.

In 2003, Oriol and coworkers [39] reported the synthesis and charac terization of new liquid crystals derived from 4-hydroxycinnamic acid (series p) in order to evaluate the influence of the central core in the mesomorphic properties. Furthermore, they have also prepared a series of materials derived from 3-hydroxycinnamic acid (series m) which series p and series m were prepared by using acid dichloride.

In 2004, Sridarala and coworkers [40] reported that the esterification of palmitic acid using WO_3 supported on ZrO_2 solid acid catalysts. An attempt has been made to delineate the structure of the super acid that offers maximum activity.

In 2004, Xiuhua and coworkers [41] showed that metal complexes (e.g., Sn, Sc, and Yb) with bis(perfluorooctanesulfonyl) amide and tris(perfluoro octanesulfonyl) methide ponytails are highly active and recyclable catalysts in the fluorous immobilized phase.

In 2004, Chirachanchai and coworkers [42] reported that they use 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI) and 1-hydroxy-1 *H*-benzotriazole monohydrate (HOBt) as a catalyst in procedure of mPEG-COOH reacts with *N*-phthaloylchitosan.

In 2005, Johanna and coworkers [43] demonstrated the applicability of the polymer-supported sulphonic acid catalyst, Smopex-101 (Smoptech Ltd., A Johnson Matthey Company), in esterification reactions and to study the esterification kinetics.

In 2005, Abd El-Wahab and coworkers [44] examined the catalytic activity and selectivity of phosphomolybdic acid supported on silica gel and modified with alkali metal hydroxides towards the gas-phase esterification of acetic acid by ethyl alcohol.

In 2005, Firdovsi and coworkers [45] reported the esterification reaction of phthalic anhydride by 2-ethylhexanol in the presence of several solid acid catalysts such as zeolites, heteropolyacid $H_4Si(W_3O_{10})_4$, and sulfated zirconia under solvent-less condition in simplification of the esterification process.

In 2005, Rabindran Jermy and coworkers [46] showed that the both of PW_{12} and SiW_{12} supported on MCM-41 as the active catalyst in the vapour phase esterification of acetic acid with 1-butanol and they found excellent catalytic activity for the protonated form of Al-MCM-41 under autogeneous pressure in the process.

In 2005, Nava and coworkers [47] reported they can be synthesized and characterized (S_{BET}, XRD, FT-IR and thermal analysis) the catalytic

activity of ZnA immobilized on the surface of a mixed silica–alumina gel obtained by the sol–gel method and previously modified by succinic acid for esterification reaction.

In 2006, Yukako and coworkers [48] reported on the simple and mild esterification of n-protected a-amino acids using approximately equimolar amounts of alcohols via tert-butoxycarbonyl esters using 1-tert-butoxy-2-tert-butoxycarbonyl-1,2- dihydroisoquinoline (BBDI) In pursuing itinterested in the use of BBDI as a tert-butoxycarbonylation reagent in the organic synthesis further it described that the novel BBDI-catalyzed esterification of n-protected a-amino acids in the presence of Boc_2O herein.

In 2006, Manisha and coworkers [49] modified silica is silica chloride (SiO_2-Cl) which has been reported to be an efficient catalyst for esterification of phosphonic/phosphoric acids.

In 2006, Bamoharram and coworkers [50] studied performance and applicability of sodium-30-tungstopentaphosphate, the so called Preyssler's anion for highly selective and efficient esterification of salicylic acid with some aliphatic and benzylic alcohols.

In 2006, Qiao and coworkers [51] reported that the application of acidic ionic liquids as catalysts in synthetic chemistry, which involves the preparation of novel 1-allylimidazolium containing acidic ionic liquids that are immobilized on modified silica gel by covalent bond and their use as recyclable heterogeneous catalysts for esterification and nitration reaction.

In 2006, Zheng and coworkers [52] reported that SBA-15 functionalized by propyl-sulfonic groups with ''single site'' was an effective catalyst which was expected to be selective toward the targeted product for the esterification of salicylic acid with dimethyl carbonate.

1.7 Research goal

Nowadays, many people use sunscreen for protecting UV radiation, as mentioned earlier, it still possesses few problems including absorption through human skin [10, 13-19] and less of the efficient UVA absorbers are found in cosmetics market. Few methods have been proposed to reduce skin penetration of sunscreen.

Curcumin is widely used in pharmaceutics and foods since it has many biological activities [23-38] such as anti-oxidant and anti-inflammatory. The research showed that modification of curcumin structure can absorb UVA radiation [53].

However, transdermal permeation of the sunscreens cannot be totally blocked. We propose the preparation of curcumin oligomer for increasing molecular weight and absorbing UVA radiation, which higher molecular weight of the resulted UV absorbing oligomer comparing to the conventional small UV filtering molecule, transdermal absorption should be considerably decreased.

The objectives of this research can be summarized as follows:

1.7.1 To synthesize curcumin oligomer and modified structure for using UVA filter

1.7.2 To investigate molar absorptivity, penetration and photostability of the synthesized materials