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STUDY OF THE IMPROVEMENT OF DYEABILITY AND OTHER FUNCTIONAL PROPERTIES OF CURCUMIN

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Study of the Improvement of Dyeability and Other Functional Properties of Curcumin

By

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A thesis submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy

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Abstract

Nowadays, the interest in natural dyes for textile application has been increasing due to the carcinogenic problems of certain petroleum-based synthetic dyes to human beings and animals and their difficulty in decomposition. In this work, curcumin was selected as a substantive natural dye which could be a potential alternative to synthetic yellow dye for textile coloration. The aims of the research are to investigate the dyeability of curcumin and to further develop useful functions and properties based on the chemical modification of curcumin.

The research work started with the extraction of the curcumin from dry turmeric powder. The curcumin dye was then applied to dye cotton and other 3 synthetic substrates using conventional dyeing methods. The colors of the dyed samples were different shade of yellow with medium color strength. Polyester fabrics and acrylic yarns dyed exhibited satisfactory results of colorfastness to laundering and other, i.e., PLA and cotton exhibited low colorfastness to laundering.

To improve dyeability and antibacterial activity of curcumin dye, the incorporation of N-phthaloyl glycine moiety into the structure of curcumin was attempted. Monoester curcumin with molecular weight of 555.53 was obtained as a major product. The modified curcumin with N-phtaloyl protecting group was investigated for its dyeability and antibacterial activity. Color of monoester curcumin on polyester fabric was greenish yellow but remained unchanged as concentration of monoester curcumin increased. Its color strength on polyester fabric compared to that of curcumin-dyed polyester fabric was lower. These dyeing characteristics were

in consistence with the occurrence of hypsochromic (blue) shift and hypochromic effect as indicated by the absorbance values of monoester curcumin. For antibacterial activity, monoester curcumin exhibited lower antibacterial activity than unmodified curcumin possibly due to the disappearance of a phenolic group.

In another chemical modification of curcumin, glycidyltrimethylammonium chloride (GTMAC) was chosen to introduce the quaternary amino moiety onto curcumin molecules via etherification reaction. The major product was characterized and identified as a quaternary ammonium cationized curcumin. As the result of the absorbance measurement, the maximum absorption wavelength of the GTMAC-modified curcumin or CurGTMAC was shifted to the region of ultraviolet radiation at 346 nm possibly due to the replacement of hydrogen atom of a phenolic group of curcumin with a quaternary ammonium group. From in vitro cytotoxicity test using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and skin irritation test according to ISO 10993-10:2010, neither skin irritation nor obvious sign of cytotoxicity to the normal human skin fibroblast cells up to 125 μg/ml was observed.

To increase the fixation between CurGTMAC and cotton, citric acid (CA) was primarily introduced to treat cotton fabric. Unreacted carboxyl group of citric acid remaining on CA-treated cotton may become ionized and subsequently react with CurGTMAC molecules in water during dyeing process. The color of dyed cotton was yellow with less saturation (C*) and more greenish tint compared to that of the unmodified curcumin-dyed cotton. The color strength (*K/S*) value at the concentration of 2 % o.w.f. of dyed cotton at the maximum absorption wavelength

was 1.95. In consequence of hypsochromic shift to near UV region with a high molar extinction coefficient (ε_{molar}) of 1,844 $M^{-1}cm^{-1}$, the treated cotton dyed with CurGTAMC was investigated for UV absorbing properties. The UPF rating of the treated cotton dyed with 1 % o.w.f. of CurGTMAC was higher than 100 according to AS/NZS 4399:1996. After 6 accelerated laundering cycles, equivalent to 30 home laundering cycles, UPF rating remained higher than 40 indicating the durability of UV-protection of the dyed cotton against accelerated laundering. With the knowledge of antibacterial property for curcumin, dyed samples were evaluated for their antibacterial property against both *S.aureus* and *E.coli*. The results showed that both curcumin and CurGTMAC provided bacteriostatic rather than bactericidal properties and CurGTMAC showed higher percentage of bacterial reduction than curcumin.

The reaction of curcumin with glycidyltrimethylammonium chloride in an aqueous system can be an indication of the successful use of renewable resources; that is curcumin and water. The use of water as a reaction media is currently gaining an importance in organic synthesis since it is more environmentally friendly and contributes less pollution to the environment compared to the use of volatile organic solvents. Moreover, the modification reaction conducted in this work could produce the modified curcumin possessing better properties in water solubility, dyeability, and antibacterial property on cotton and gaining additional UV protective property. It also can be an initiation to develop natural dyes to be more valuable and useful than their original structures.

List of Publications

Conference Presentation

S. Boonroeng, L. He, K. Srikulkit, and J.H. Xin. (2013). Modification of Curcumin and Its Dyeability to Textile Fiber, *Abstracts of papers of the 15th Asian Chemical Congress 2013*, 19-23 August , Singapore.

Journal Article

Supannee Boonroeng, Kawee Srikulkit, John H. Xin, and Liang He. (2015). Preparation of a Novel Cationic Curcumin and Its Properties Evaluation on Cotton Fabric, *Fibers and Polymers*, 16(11), 2426.

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Chapter 1: Introduction

1.1 Background

Before the advent of synthetic dyes, the natural dyes were the only source for textile coloration. They have met people's demand on "living in color" since an ancient time (Bechtold & Mussak, 2009). Although the dyeing processes of natural dyes were considered systematic, they were comparatively complicated and consumed longer time. Their color shades and depth were limited. Moreover, their properties such as color fastness were inferior to those of synthetic dyes. Consequently, their uses were dramatically declined, remaining only in some specific areas such as craftsmanship (Hill, 1997).

Recently, the world has encountered serious problems on health and environmental issues. An important awareness is raised from the problems of certain azo synthetic dyes which are toxic and allergic to human beings and harmful to the environment. More than 100 azo dyestuffs, mostly direct and acid dyes, have been prohibited for the coloration of textile materials (OEKO-TEX@ Association, 2015). These dyes are either manufactured from amine intermediates designated as carcinogenic compounds by Oeko-Tex standard 100 or European Union Directive 2002/61/EC or being sources of undesirable chemicals releasing from reductive cleavages. Moreover, certain disperse dyes have been identified as substances responsible for textile dermatitis. They are considered as allergenic dyes (Dawes-Higgs & Freeman, 2004). In consequence, natural dyes are recurrent. Compared with synthetic dyes, natural dyes have lower toxicity and more compatible to both human and the environment. Certain natural dyes such as quercetin, brazilin, and curcumin have been used not only for textile coloration but also in other applications especially in therapeutics

(Choi & Kim, 2008; Edwards, *et al.*, 2007; Ravindran, 2007). Thus, natural dyes can be a potential alternative for consumers who call for healthy and environmentally friendly products. Among natural dyes, the predominant one is curcumin, a common name for a natural yellow dye, and its derivatives. Various works on curcumin have been published (Dahl, *et al.*, 1989; Santis & Moresi, 2007; Goel, *et al.*, 2008; Sandur, *et al.*, 2007). Apart from being a natural dye for textiles, curcumin has been used as food coloring agents (Jayaprakasha, *et al.*, 2005), photo initiators (Martin, *et al.*, 1989), and therapeutic medicine (Das, *et al.*, 2010).

In textile dyeing process, curcumin provides a bright yellow color on cotton, silk, and wool, but its water solubility is poor and its color fastness seems to be unfavorable (Siva, 2007). Although its dyeability and color fastness could be improved with mordanting processes, most mordants used are metallic compounds such as alum, ferrous sulfate, and copper sulfate. It has been subjected to certain environmental concerns. Several attempts have been reported to avoid the use of these mordants. At the same time, certain natural products have been introduced, which are called green, natural or bio mordants such as chitin, chitosan, and a substance from certain plants (Poornima & Devi, 2007).

The main structure of curcumin contains benzene rings and conjugated diene. It has only two phenolic groups attached to the main structure as shown in the Figure 1.1 below. They are responsible for water solubility property. Thus, curcumin has low water solubility. Certain works have been attempted to improve this property by modifying the structure of curcumin.

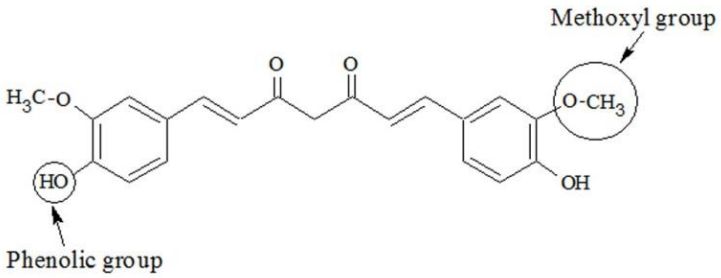


Figure 1. 1 Chemical structure of curcumin**.**

These phenolic groups can react with other chemicals. For example, it can be esterified with a photoactive diazo oxide to become more efficient photoactive products. Its phenolic group can also react with an anhydride or an alkyn to be curcumin azide and curcumin alkyn, respectively (Shi, *et al.*, 2007). These two compounds can further react with each other to be a curcumin dimer possessing more potential therapeutic properties. Moreover, a phenolic group of curcumin can be covalently linked to certain amino acids such as glycine and other chemicals to obtain potentially more effective biological properties (Mishra, *et al.*, 2005). Accordingly, the chemical modification of curcumin with glycine and glycidyltrimethylammonium chloride was conducted to improve its properties and potential for dyeing applications.

Considering the unique properties of the natural dyes, this work is aimed at the investigation of their applications to textile fibers, with special focus on curcumin and its related types. The essence of work was approached mainly due to the following information:

a) Certain natural dyes have been reported as potential therapeutic agents, especially curcumin from turmeric (*Curcuma longa L.*). It is probable to exhibit both dyeing and functional finishing properties;

b) Metal salts still play an important role in enhancing dyeability of certain natural dyes on cotton but their use should be minimized or replaced by more environmentally friendly substances;

c) Modification methods of certain natural dyes such as curcumin with small biomolecules, i.e., amino acids or other compounds are practical;

d) Functional textiles which are currently favorable may be obtained from the use of chemically modified curcumin.

1.2 Research Objectives

Many natural materials contain colorants and other components that are useful for various applications such as food preservation, pharmacology, and textile coloration. The exploration of these natural materials together with their potential applications in a particular field as well as the improvement of their properties is not an easy task. In this research work, the study of the natural dyes for both textile coloration and functional finishing was conducted. The objectives of this research are as follows:

(1) To explore the natural dyes and their coloration of textiles without the use of metal mordants with the focus on curcumin and its derivatives.

(2) To investigate the antimicrobial effects of curcumin and its derivatives dyed on textile substrates.

(3) To improve the dyeability and dyeing properties of natural dyes, particularly curcumin towards cellulosic with the ecologically friendly chemical modification.

(4) To further improve the antimicrobial effects of curcumin and its derivatives dyed on textile substrates with the ecologically friendly

chemical modification.

(5) To study other properties of textiles dyed by curcumin and its derivatives.

1.3 Research Originality, Significance, and Value

As natural dyes are resurfaced due to negative impact of certain synthetic dyes on human beings and the environment, curcumin can be a noteworthy alternative for synthetic yellow dye. However, dyeing application of curcumin has limitations due to its poor water solubility in neutral and acidic conditions and less affinity to cotton fibers. Although dyeing properties of curcumin on cotton fibers, such as dyeability and colorfastness, and antibacterial property can be enhanced with the use of metallic mordants, a substantial amount of metallic mordants used in dyeing process may pose serious effluent disposal problems (Samanta & Konar, 2011). To improve dyeability and other properties, i.e., antibacterial property, chemical modification of curcumin with glycidyltrimethylammonium chloride was first time conducted in this work in an aqueous alkaline solution. The use of water as a reaction media for the modification reaction of curcumin should gain an importance in view of the following considerations: using water and curcumin as renewable materials, relatively safe process due to the absence of hazardous chemicals and solvents, and less contribution to environmental pollution compared to the use of volatile organic solvents. The novel modified curcumin exhibited better dyeability and antibacterial properties with an additional UV protective property on cotton fibers; that is an indication of value-added to the applications of natural dyes as a result of the chemical modification. In addition, this work can be an initiation to

chemically develop colorants to function more productively as a textile dye and functional finishing agent as well as being friendly to both human being and the environment.

1.4 Structure of the Thesis

The thesis is divided into 9 chapters. The first chapter is an introductory chapter which provides the essential background of natural dyes mainly focused on curcumin. Research objectives are also explained in this chapter followed by the research originality, significance, and values.

The second chapter presents the review of the relevant literature starting from principal knowledge related to natural dyes. Then the solid background in chemical structures and properties of curcumin is provided including development of its chemical modification and utilization.

The third chapter describes materials and chemicals used for the experiments. The explanation of important instruments and machines is given in this chapter. All standard test methods are also briefly reviewed herein.

The fourth chapter describes the methodology to isolate curcumin from turmeric raw material. The potential applications of curcumin in dyeing on natural and synthetic substrates: cotton, polyester, poly(lactic acid), and acrylic substrates are presented.

The fifth and sixth chapters describe two different approaches in synthesis of curcumin derivative. In the fifth chapter, the reaction is involved with N-protected amino acid, while the use of glycidyltrimethylammonium chloride (GTMAC) is exhibited in the sixth chapter. The characterization of the each

modified curcumin using mass spectrometer, NMR, and UV-visible spectrometer is presented in the relevant chapter. The possible mechanism for individual reactions is also proposed and discussed.

The seventh chapter highlights the important properties and applications of the GTMAC-modified curcumin.

The eighth chapter is the conclusions and recommendations of the thesis. The ninth chapter is the references.

Chapter 2: Literature Reviews

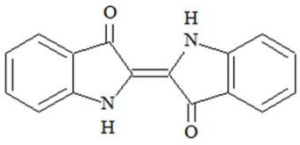
2.1 Natural Dyes and Their Current Status

2.1.1 Definition and Classification of Natural Dyes

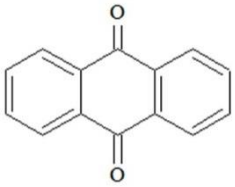
Natural dyes are the colorants derived from plants, animals, and minerals. Most of them are obtained from various parts of plants: roots, barks, stems, leaves, fruits, seeds, and flowers (Vankar, 2000).

Grouping of natural dyes is diversified. For example, they can be classified based on sources, methods of application, chemical structures, or colors. Based on sources, natural dyes are divided into 3 groups: natural dyes from plants, animals, and minerals. According to application methods, 3 categories are well known: substantive dyes, additive or mordant dyes, and vat dyes (Fereday, 2003). Substantive dyes can dye directly to the substrate without the use of mordants. They possess certain polar functional groups capable of forming chemical bonds mostly ionic bonds with fibers to be dyed. Curcumin from turmeric, bixin from annatto, and berberine are examples of this category. Additive dyes need a mordant to fix them to fibers. Vat dyes are water insoluble. They must be converted into a soluble form and then changed back to the original form after dyeing stage. Another system widely used to group natural dyes is related to their chemical structures. They are illustrated as indigo, anthocyananidins, anthraquinone, naphthoquinone, carotenoid, flavonoid, dihydropyran, and chlorophylls (Siva, 2007). Certain chemical structures are shown here.

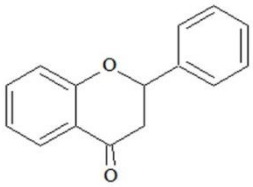
Indigo



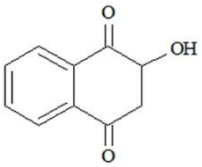
Anthraquinone



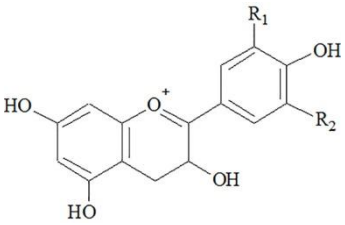
Flavone



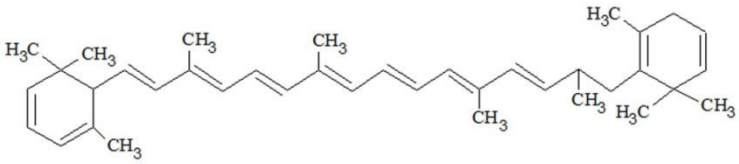
α-hydroxynaphthoquinone



Anthocyanidins



β-carotene



Hematein

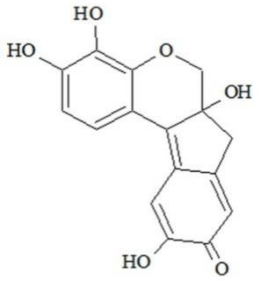


Figure 2. 1 Molecular structures of natural dyes categorized by their chemical structures (Siva, 2007).

2.1.2 The Current Status of Natural Dyes

In earlier decades, natural dyes have gained much attention from various groups of people including scientific researchers, dye plants farmers, natural dyes and pigments producers, and textile companies. With the gradual exhaustion of fossil resources, renewable resources are increasingly emphasized as the alternatives. This consideration together with human health and environment concerns in use and disposal of certain synthetic dyes has evoked people's attention back to natural dyes and thus their significance has been brought up. The recent interest in natural dyes is not only for studying historical, archaeological and heritage aspects, but also for economic and ecological purposes. Certain crucial issues related to natural dyes were raised for discussion at the International Symposium and Exhibition of Natural Dyes in France in 2011 such as sustainable development of the production and uses of natural colorants and environmentally friendly production methods. To fulfill customer requirements, the challenge of scaling production up should be overcome. Moreover, interdisciplinary scientific research was suggested to be called for to search worldwide possibilities in all sources and aspects of natural dyes and pigments to serve this purpose. This

included the study and identification of traditional dye sources, the exploration of new possible sources particularly among the waste products of plants with other primary uses, eco-friendly production technology, and the investigation related to the simplification of their uses with optimized advantages in industry and fashion, as well as their marketing and promotion (Cardon, 2010).

Natural dyes cover a wide range of color shades with the use of mordants (Vankar, 2000) or mix and match approach (Kumbasar, 2011). Many natural dyes have been specified with regards to their chemical classes published in the Colour Index. As mentioned previously, the main group of natural dyes is derived from various parts of plants such as flowers (saffron), petals (Dyer Thistle), root (madder plant), wood (Venetian Sumach), leaves (various species of *Indigofera*), sheaths (Great Millet grass), and bark (Kasila tree) (SDC & AATCC, 1971).

To improve the utilization of natural dyes, diversified studies have been conducted including optimization of extraction methods, improvement of dyeing techniques, and exploration of new sources. For example, Ali, *et al.* (2009) found that the dye extracted from Henna leave in alkaline solution provided higher color strength on cotton fabric compared to the result obtained from distilled water extract. To obtain a better yield, De Santis and Moresi (2007) used 4 organic solvents (ether, acetone, ethanol, and methanol) and distilled water to extract dye from root of common madder (*Rubia tinctorum*) and compared the extraction results. The best result was from the use of methanol. Moreover, certain techniques have been used to optimize the extract yield of natural dyes such as the use of an ultrasound. Sivakumar, *et al*. (2009) compared

the extraction efficiency of ultrasonic energy with conventional heating method and found that the efficiency of ultrasound method was higher. Thus, ultrasound method could be used as an alternative heating source for natural dye extraction. Certain parameters of using ultrasound to extract natural dyes also have been studied such as ultrasonic output power, time, pulse mode, and effect of solvent system. Albu, *et al.* (2004) successfully used ultrasound to increase the efficiency of organic solvent extraction of carnosic acid from the herb *Rosmarinus officinalis*.

In order to support the need of natural dyes, new sources have been explored in many regions of the world including Europe, South America, and Asia. Certain plants commonly found in the mentioned regions could be potential sources of natural dyes. In India, Siva (2007) reported that there are more than 450 plants showing their capability of yielding dyes and many of them are still being further investigated. In central Italy, weld cultivation was carried out to study their agronomic characteristics in order to obtain maximum yield of luteolin, a common name of a natural yellow dye (Angelini, *et al.*, 2003). In Europe, recultivation of certain plants in large scale was cited such as madder in Netherland, woad in France (Cardon, 2010). Moreover, certain plants were selected for large scale production of natural dyes such as weld (*Reseda luteola L.*), dyer's broom (*Genista tinctoria L.*), and dyer's chamomile (*Anrthemis tinctoria L.*). In addition, waste and by-products from certain industries could be potential sources of natural dyes. For example, anthocyanin natural dye could be produced from waste of food and wine processing in France. Timber industry was also another interesting source for natural dyes (Cardon, 2010). Natural dyes can be also obtained from fungus and bacteria. Anthraquinone dyes isolated

from the fungus *Fusarium oxysporum* were used to dye wool fabric with exhaustion dyeing method (Nagia & EL-Mohamedy, 2007). Emodin and dermocybin are two naturally occurring anthraquinones isolated from the fungus *Dermocybe sanguine*. They were applied to wool, polyamide, and polyester fibers using different dyeing techniques (Raisanen, 2001).

Since color shades from natural dyes are soft and unique with soothing sensation, several attempts have been tried to improve their dyeing ability. Certain dyeing techniques have been developed. Vankar, *et al.* (2007) substituted metal salts with the mixture of enzyme and tannic acid in the dyeing process of silk and cotton with two natural dyes, techtona and catechu. It was resulted that enzyme-tannic acid complex could increase dyeability and made the process more practical for industrial application. Komboonchoo and Bechtold (2009) presented the concept of hybrid dyeing process. Certain modified natural dyes were applied in combination with others. Drivas, *et al.* (2011) synthesized disperse dyes by alkylation of hydroxyl group in certain natural anthraquinone dyes, alizarin and purpurin. Paul, *et al.* (2005) dyed wool fabrics with C.I. Natural Orange 2, a water insoluble natural dye, using an oil-in-water (O/W) microemulsion as a dyeing media.

Moreover, there was an attempt to set up the standardization method for natural dyes. Bechtold, *et al.* (2007) selected Canadian golden rod to develop the standardization method for yellow natural dyes. The distilled water-extracted Canadian golden rod dye solution was dyed on cotton fabric and wool yarn with the use of 3 mordants. Two parameters of the dye extract, its absorbance and total phenolic component, were measured in order to search for correlations with

concentrations of the dye. However, these two parameters did not show resolving power strong enough to establish a good correlation due to several reasons such as the influence of certain insoluble substances from the extract.

The study of natural dyes has not been conducted only for textile coloration. A considerable number of research works have been reported for their intrinsic medicinal properties. For examples, quercetin, a common name for C.I. Natural Red 1, has been reported as an anitoxidants and it can be used as adjunct therapy to control blood pressure in hypertensive individuals (Edwards, *et al.*, 2007). Brazilin is a major component extracted from *Caesalpinia sappan L*. It can be oxidized to brazilein, a common name of a natural red dye (C.I. Natural Red 24). Brazilin has been used as an analgesic and anti-inflammatory agent to cure emmeniopathy, sprain, and convulsions (Hu, *et al.*, 2003). The medicinal properties of curcumin have been widely investigated. Sandur, *et al.* (2007) examined the difference in regulation to anti-inflammatory and anti-proliferative responses among curcumin and its analogs. Anand, *et al.* (2008) reported that curcumin has been linked with suppression of several diseases and injuries of body tissues such as inflammation and diabetes. In order to enhance the utility of curcumin, the review of the status of all approaches to generate and to improve bioavailability of curcumin was also written in their paper including the summary of curcumin analogues with reference to the properties.

2.1.3 The Characterization of Natural Dyes

Most natural dyes are from different parts of plants. They can be extracted by several techniques. The widespread uses are aqueous extraction, organic solvent extraction, and steam distillation.

Aqueous extraction is the most commonly used to obtain textile dyeing natural dyes. Its condition can be neutral, acidic, or alkaline depending on properties of natural dyes. Chairat, *et al.* (2007) obtained the optimum amount of a natural dye for dyeing cotton and silk yarns from extracting the dried fruit hulls of Mangosteen, *Garcinia mangostana Linn* with 15 % (w/v) aqueous citric acid solution at 80 ^{o}C for 1 hour. Ali, *et al.* (2009) found that alkaline aqueous condition was favorable for extracting a natural dye from Henna leave. Contrarily, aqueous solution extracted from turmeric powder showed the highest color strength, when dyed at pH 6 on cotton fabric (Bhatti, *et al.*, 2010). In addition, Sivakumar, *et al.* (2009) reported that the efficiency of an aqueous extraction was higher with the use of ultrasound heating rather than conventional heating coupled with magnetic stirrer.

Certain organic solvents have been frequently used to extract natural dyes especially in food and pharmaceutical industries. Most organic solvents are short chain alkyl alcohols. Nishiyama, *et al.* (2005) used ethanol and hexane to extract the volatile oil (sesquiterpinoids) and coloring agent (curcuminoids) from turmeric rhizome for diabetic therapy study. Braga, *et al.* (2003) extracted curcuminoids from turmeric powder using 4 different methods: hydrodistillation, low pressure solvent extraction, Soxhlet extraction, and supercritical carbon dioxide. The highest amount was obtained with the mixture of ethanol and isopropyl alcohol using a Soxhlet system. For natural dyes, the use of organic solvent was also reported. Bajpai and Vankar (2007) used methanol to extract a natural dye from *Mahonia napaulensis D.C.* Leaves at 75 ^{o}C. The extract showed both dyeing and antifungal finishing properties when dyed on cotton fabric.

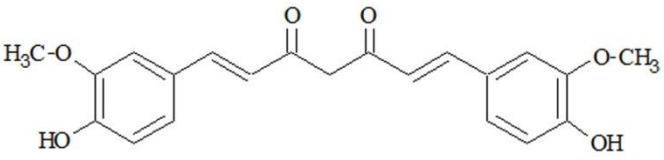
Last decade, supercritical fluids have been introduced as an alternative to the use of organic solvents. They are likely to gain more attention for extracting organic compounds from natural plants since they have high solvating powers with safer and more eco-friendly to the environment. The most commonly used substance is carbon dioxide. Petrović, *et al.* (2010) used critical carbon dioxide to extract cuticular waxes and resins out of the tubular, head, and ligulate flowers of *Calendula officinalis L*. before extracting essential oils with steam distillation. Highly purified turmeric oil was successfully extracted from turmeric powders with supercritical carbon dioxide without any use of co-solvent. Chang, *et al.* (2006) also used supercritical carbon dioxide to extract turmeric oil from *Curcuma longa Linn* at 60 ^{o}C. Although this extraction method is remarkably interested, it requires expensive equipment.

2.2 Curcumin – Natural Yellow Dye

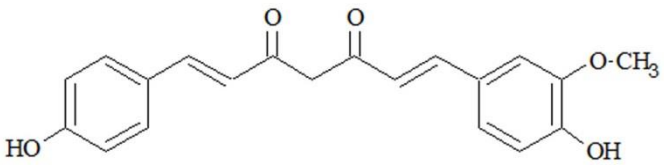
Curcumin is a common name of C.I Natural Yellow 3, one of the most well-known natural dyes as a bright yellow coloring agent. Its molecular formula as indicated in The Colour Index is $C_{21}H_{20}O_6$. Curcumin occurs naturally in the rhizome of *Curcuma longa L.*, commonly known as turmeric, a perennial herb and indigenous to tropical Asia (SDC & AATCC 1971). It can be isolated using various procedures. Sachan and Kapoor (2007) extracted curcumin by steeping turmeric powder in water for overnight. The temperature of the mixture was then increased and remained constant at 90 ^{o}C for 5 hours to extract curcumin. The aqueous extract of curcumin was made more concentrated using spray drying method and then dried at 45 ^{o}C. Manzan, *et al.* (2003) identified the suitable conditions for steam distillation and solvent extraction methods in order to

achieve the maximum yields of volatile oil and colorants from turmeric rhizomes. The products obtained from both methods were the mixture of volatile oil and curcuminoids, expressed as curcumin, demethoxycurcumin, and bisdemethoxycurcumin. Nishiyama, *et al.* (2005) conducted the extraction of fine turmeric powder using two organic solvents, ethanol and hexane. The extract components from individual solvent were different. The ethanol extracts yielded curcuminoids and sesquiterpenoids volatile oil, whereas the products from the hexane extraction were only sesquiterpenoids. To obtain curcuminoids, hexane extraction was primarily conducted and its crude extract was then further isolated by ethanol. Nazi, *et al.* (2010) isolated volatile oil and curcuminoids from turmeric rhizomes using hydrodistillation and Soxhlet extraction methods, respectively. Stankovic (2004) also concluded that curcumin could be isolated by extraction process and followed by crystallization to purify the extract.

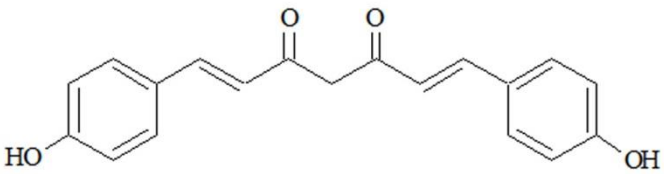
The curcuminoids extract products are essentially consisting of curcumin and its 3 derivatives, demethoxycurcumin (curcumin II), bis-demethoxycurcumin (curcumin III), and lastly identified cyclocurcumin (Ravindran, 2007). Commercial curcumin contains curcumin I (~77 %), curcumin II (~17 %), and curcumin III (~3 %) as its major components (Stankovic, 2004).



Curcumin I



Curcumin II



Curcumin III

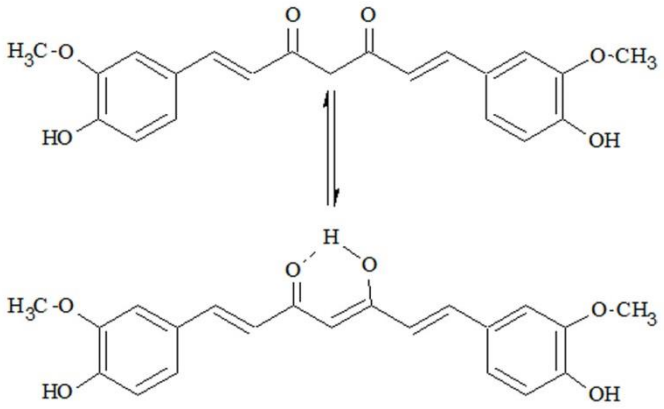
Figure 2. 2 Chemical structures of curcumin (Ravindran, 2007).

2.3 Curcumin Structures

Curcumin is identified as (1E, 6E)-1,7-Bis-(4-hydroxy-3-methoxyphenyl)-hepta-1,6-diene-3,5-dione or diferuloylmethane with the formula weight of 368 (Goel, *et al.*, 2008). Its derivatives are 1-(4-Hydroxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-hepta-1,6-diene-3,5-dione or p-hydroxycinnamoylferuloyl methane with formula weight of 338 and 1,7-Bis-(4-hydroxyphenyl)-hepta-1,6-diene-3,5-dione or p,p-dihidroxydicinnamoylmethane with formula weight of 308. Their chemical formulas' are $C_{20}H_{18}O_5$ and $C_{19}H_{16}O_4$, respectively (Stankovic, 2004).

Curcumin can exhibit a keto-enol tautomerism having a C=O and a C-OH groups for enolic form and two C=O groups for β diketonic form as shown in Figure 2.3. In solid phase, curcumin is in diketonic form. In solution, it is in a predominant diketo form under acidic and neutral conditions and in the enol form in alkaline solution (Stankovic, 2004).

diketo-form



enol-form

Figure 2. 3 Keto-enol tautomerism of curcumin (Stankovic, 2004).

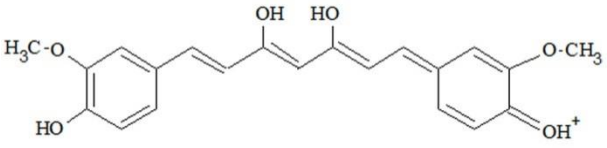
2.4 Properties of Curcumin

2.4.1 Solubility Properties

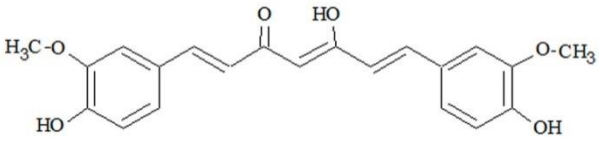
Curcumin is slightly soluble in hot water but soluble in 0.1 M sodium hydroxide and stable in this condition for 1-2 hours. Normally, curcumin is degraded under alkaline aqueous conditions to ferulic acid and feruloylmethane (Stankovic, 2004). Under neutral condition, it can be stable at high temperatures (Sogi, *et al.*, 2010; Wang, *et al.*, 2009). Curcumin powder has a yellow-orange color. In aqueous solution, its color is yellow at pH 1-7. At pH <1 or > 7.5, the color will change to be red (Stankovic, 2004).

Curcumin is highly soluble in polar organic solvents such as acetone, ethanol, methanol, and toluene but slightly soluble in aliphatic or alicyclic organic solvent such as hexane and cyclohexane (Ravindran, 2007). Curcumin in different solvents has different maximum absorption wavelengths such as at 430 nm in methanol and ethanol and 415-420 nm in acetone (Goel, *et al*., 2008).

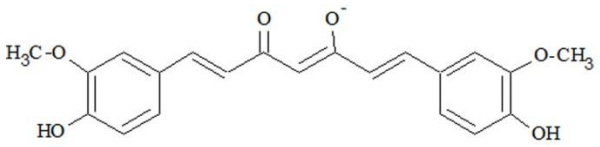
pH <1



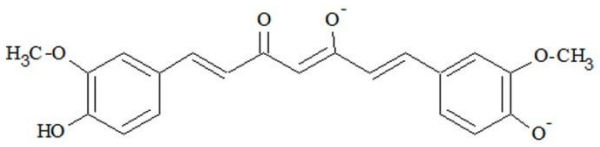
pH 1-7



pH 7.8



pH 8.5



pH 9.0

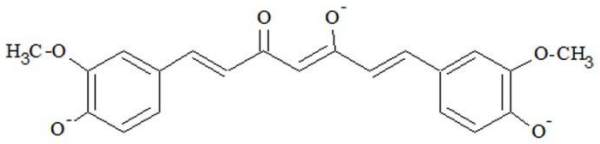


Figure 2. 4 Chemical structures of curcumin at different pH values (Stankovic, 2004).

2.4.2 Dyeing Properties

Curcumin is a yellow–orange powder. It is a main active chemical constituent of turmeric. Its structure has two symmetrical sequences of conjugated double bond systems of feruloylmethane or 4-(4-Hydroxy-3-methoxyphenyl)-3-buten-2-one. These systems contribute curcumin to absorb visible light in the region around 420-440 nm rendering dyed substance yellow color (Dahl, *et al.*, 1989). Curcumin can also give fluorescence emission but with a low quantity yield (Patra & Barakat, 2011). Nevertheless, the quantity is high enough to work as a fluorescent agent for observing the biological mechanisms at molecular level (Adhikary, *et al.*, 2009). Curcumin can be combined with other natural dyes such as indigo and saffron to impart different color shades (Gupta & Balasubrahmanyam, 1998). The incorporation of mordants can enhance dyeing properties and color fastness of curcumin dyeing particularly on cotton and a variety of colors are produced with different mordants used such as orange yellow with alum (SDC & AATCC, 1971). To achieve maximum dyeing properties, various experiments have been attempted to dye curcumin on both natural and synthetic fibers. Umbreen, *et al.* (2008) obtained the optimum color strength and uniformity from dyeing curcumin on cotton fabric at 70 ^{o}C with sodium sulfate. Its color fastness to washing and light was poor but improved with mordants. Bhatti, *et al.* (2010) improved color strength and fastness properties of curcumin-dyed cotton with gamma irradiation exposed on both dyeing substrate and turmeric powder. Tsatsaroni, *et al.* (1998) used enzymes and a protein to treat cotton and wool substrates before dyeing with curcumin to gain better results. Adeel, *et al.* (2011) exposed UV irradiation to both turmeric powder and cotton substrate to obtain better dyeing results and properties.

In coloration of synthetic fibers, curcumin has been reported for its use in dyeing of modified acrylic fiber, nylon 6,6, polyester and polylactic acid or PLA fibers. El-Shishtawy, *et al.* (2009) successfully dyed amidoxime-modified acrylic fibers with curcumin. The fibers with higher amount of nitrogen contents showed better dyeing results due to higher proportion of amorphous region from amidoxime groups. The best dyeing result was achieved when dyeing at pH 5 and at the temperature of 100 ^{o}C. At this pH, curcumin is likely in a favor of keto-form at equilibrium providing physical effects in dyeing similarly to disperse dyes. Mirjalili and Karimi (2013) dyed nylon 6, 6 fabrics with curcumin under acidic condition at the temperature of 100 ^{o}C. Three mordants were used by individually mixing with curcumin solution before dyeing. The dyed fabrics exhibited good color strength and antibacterial activity with durability to washing.

Kerkeni, *et al.* (2012) carried out air atmospheric plasma and UV excimer treatments on polyester fabrics before dyeing with curcumin and compared dyeing results with untreated fabrics. It was found that both eco-technologies enhanced dye uptake of curcumin on polyester fabric possibly due to an increase in hydrophilicity at the surface of the polyester fabric. However, only the hydrophilic species obtained from UV excimer treatment could resist and remain effective during dyeing at high temperature with pressure.

Dyeing of curcumin was also evaluated on hydrophobic fibers. Sriumaoum, *et al.* (2012) performed a water solubility test of curcumin and other natural dyes before conducting dyeing experiments on poly(lactic acid) and polyethylene terephthalate. The results showed that dyes with poor water solubility exhibited

higher degree of exhaustion and color strength on these two fibers including curcumin. At room temperature, curcumin is slightly soluble in water, only 0.7 % (w/w) (Sriumaoum, et al., 2012) which is close to the solubility parameters of polyester and poly(lactic acid) fibers (Suesat, *et al.*, 2011). In dyeing of PLA, an aliphatic polyester, the temperature has to be lower than the dyeing temperature of polyester in order to avoid hydrolysis especially when conducted under aqueous phase. The optimal dyeing conditions should be at the temperature of 110-115 ^{o}C, pH 4.5-5 for 15-30 minutes.

2.4.3 Biological Properties

Applications of curcumin as folk or Ayurvedic medicine have been specified over the centuries in various parts of the world, especially in India and China (Thangapazham, *et al.*, 2013). For examples, it is used to relieve abdominal pain problem in China and to treat inflammation or external injury in India *(Goel, et al.*, 2008).

Many scientific studies on curcumin have been focused on its potential in pharmacological activities to human beings as an anti-cancer agent (Majhi, *et al.*, 2010), anti- mutagenicity (Parvathy, *et al.*, 2010), antioxidant (Jovanovic, *et al.*, 1999), anti-parasite (Changtam, *et al.*, 2010), anti-inflammatory and anti-proliferative (Sandur, *et al.*, 2007), antimicrobial (De, *et al.*, 2009). For examples, Majhi, *et al.* (2010) reported that curcumin could function as an anti-cancer agent by modulating protein kinase c (PKC) activities. PKC is a group of enzymes that play a central role in cellular signal transduction. Moreover, its synthesized derivatives also showed the ability toward cancer treatment. Parvathy, *et al.* (2010) concluded that curcumin and its conjugate with amino

acid had anti-mutagenesis to bacteria, Salmonella typhimurium TA 98 and TA 1531.

In textile applications, the study of curcumin on antimicrobial properties has been found. Han and Yang (2005) dyed wool fabric with curcumin to study its antimicrobial activity and to investigate the relationship between concentration and antimicrobial ability. The results showed that curcumin had both dyeability and antimicrobial ability on wool and its color strength was correlated with bacteria inhibition rate. Ammayappan and Moses (2009) compared antimicrobial activity among aloe vera, chitosan, and curcumin. All natural materials were applied both individually and in combination with another material on cotton fabric. The results indicated that all of them exhibited antimicrobial activity in the order from high to low as follows: aloe vera > curcumin > chitosan. The potential of curcumin was from phenolic groups capable of forming hydrogen bonds with hydroxyl groups of cotton.

Reddy, *et al.* (2013) applied a series of curcumin concentrations to cotton fabrics and investigated antimicrobial activity on the dyed fabric. It was discovered that curcumin could exhibit its antimicrobial activity on cotton fabric even at a very low concentration against both Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria but its activity was better against *S. aureus*. Curcumin-dyed cotton had durability to home laundering but interior to curcumin-dyed wool.

2.5 Bacteria and Mode of Action of Antibacterial Agent

Bacteria are the oldest and the most abundant living organisms. They have three basic shapes with the same basic structure: bacillus with straight and rod-shaped, coccus with spherical-shaped, and spirillus with long and helical-shaped. Their

structure mainly consists of cell wall, cell membrane or cytoplasm membrane, and cytoplasm as shown below.

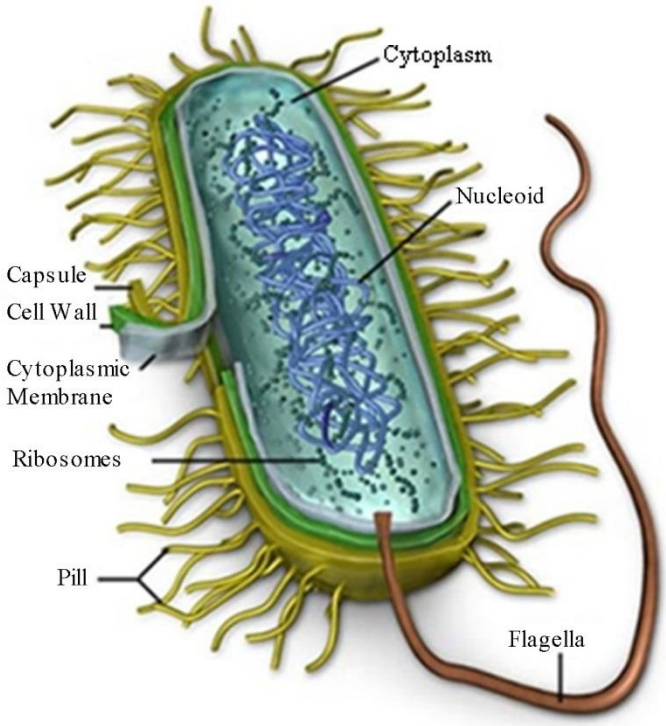


Figure 2. 5 Basic diagram of the structure of bacterial cell (http://micro.magnet.fsu.edu/cells/bacteriacell.html).

Cell wall is located outside of the cell membrane. It composes of peptidoglycan, a network of polysaccharide molecules. The characteristics of peptidoglycan vary among bacteria types. Gram-positive bacteria have thicker cell wall than Gram-negatives. Cell wall has functions to maintain shape of the cell, provide strength and protect cell from swelling and rupturing.

The inner structure from cell wall is cell membrane or cytoplasmic membrane or plasma membrane. It consists of phospholipid, proteins, and carbohydrate. Its structure is stabilized by hydrogen bridges and hydrophobic interactions.

Additionally, cations such as Mg^{2+} and Ca^{2+} are located inside cell membrane and they help to stabilize the membrane by forming ionic bonds with negative charges of the phospholipids. Cell membrane has selective permeability properties and can regulate the transfer of substances, solutes and metabolites, including removal of body wastes in and out of the cell cytoplasm.

Another important structure of bacteria is cytoplasm. It is a fluid or an aqueous gel enclosed within the cell membrane. It contains a wide variety of enzymes and molecules including spherical ribosomes. Functions of cytoplasm are related to the growth, metabolism and replication of bacteria. Ribosomes are the primary structural components in cytoplasm. Their structures consist of proteins and RNA molecules. They are sites for synthesizing protein. Another component in cytoplasm is chromosome. It is a single circular molecule of DNA which holds all genes of bacteria. Many bacteria have a second chromosome called plasmid. Cell chromosome is the center to control genetic of the cell which determines all the properties and functions of bacteria (Panno, 2005; Raven & Johnson, 1996; Schumann, 2006; Silhavy, *et al.*, 2010).

Mode of Action of Antibacterial Agents

Since bacteria are prokaryotes, they have basic structures different from plants, animals, and fungi which are eukaryotes. The ultimate aim of all antimicrobial agents must be only to kill pathogenic bacteria or inhibit the growth and multiplication of bacteria without affecting or being toxic to any cells of bacteria host. Therefore, an attack site should be the structure which is unique for bacteria and does not exist in host cell such as cell wall.

Modes of action of antimicrobial agent can be mainly classified according to target sites within the bacterial cells as follows:

a) inhibition of cell wall synthesis

   Peptidoglycan layer is the critical attack site. Loss or damage of this layer can destroy the rigidity of bacterial cell wall resulting in cell death. Any prevention or interference of peptidoglycan synthesis can weaken cell wall and cell subsequently undergoes lysis.

b) inhibition of cell membrane function.

   Certain compounds such as quaternary ammonium salts can diffuse through cell wall of bacteria and interrupt membrane potential to release ions and other small constituents into the environment that cause cell death.

c) inhibition of ribosome function.

   Certain antimicrobial agents can bind to a protein in ribosomes and cause ribosomes to misread genetic code of bacteria or induces the formation of aberrant and nonfunctional complexes.

d) inhibition of nucleic acid synthesis.

   DNA of bacteria can be prevented from replication or functioning as a proper template.

e) inhibition of other metabolic processes such as folic acid or mycolic acids syntheses (Kaplan, *et al.*, 2011; Neu & Gootz, 1996; Ye, *et al.*, 2010).

2.6 The Chemical Modification of Curcumin Structure

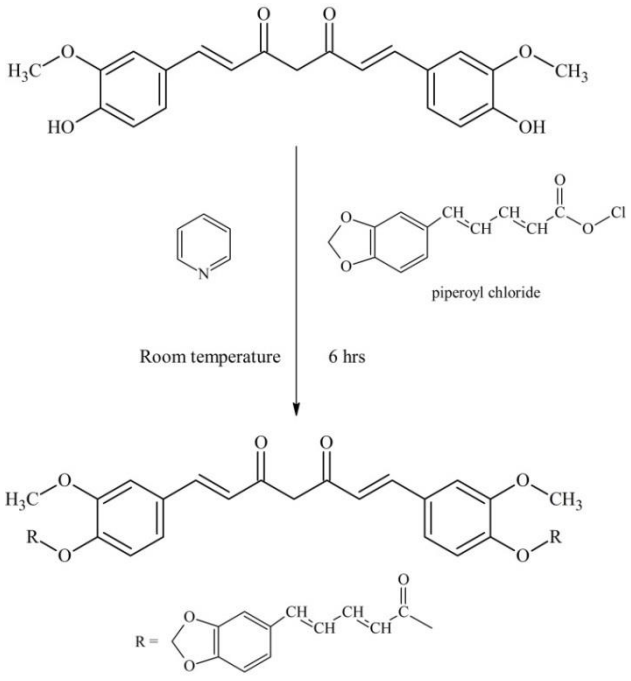
Curcumin is one of the most popular natural yellow dyes. Its function is not only as a natural dye, but also as food coloring agents, photo initiators, and therapeutic medicine. According to its structure, its water solubility is poor causing certain difficulty in use. For example, it is not a good coloring agent for water-based food products (Parvathy, *et al.*, 2010). It also has an effect to the therapeutic ability. Accordingly, the chemical structure modifications of curcumin have been carried out by researchers mostly found in food and biomedical areas.

2.6.1 Modification of Curcumin with N-Protected Amino Acids

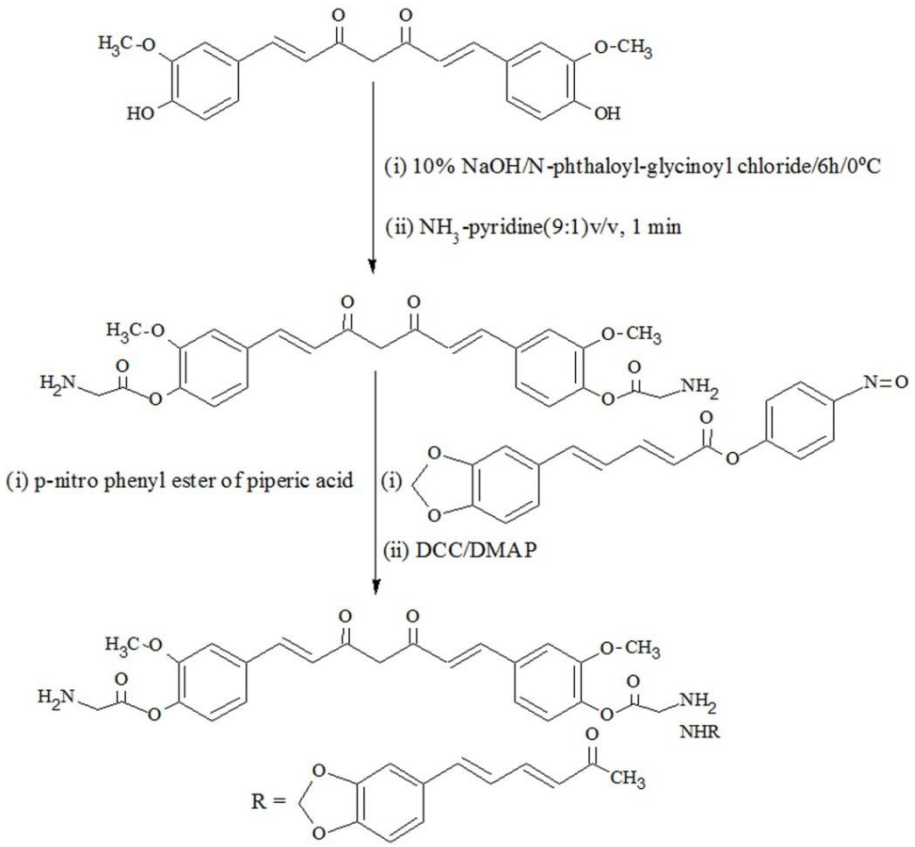
The modification of curcumin structure to enhance water solubility is possible because it contains phenolic groups capable of undergoing certain chemical reactions such as an esterification with a carboxylic acid. The esterification reaction with certain biomolecules has withdrawn attentions from scientific researchers. These molecules include amino acids, dipeptide, fatty acids, and certain organic acids obtained from natural products. The reaction was carried out mostly in different organic solvents with the presence of dehydrating agents and/or catalysts.

Mishra, *et al.* (2005) conducted the synthesis of curcumin bioconjugates for better bioavailablility by covalently linking curcumin with piperic acid and glucose derivatives. With piperic acid, the reaction was carried out via two alternative routes. The first route was the direct esterification between phenolic groups of curcumin and carboxyl groups of piperic acid chloride as seen in Scheme 2.1. The other was comprised of two steps. Curcumin was primarily

esterified by an amino acid and then further linked with piperic acid via amide linkage as seen in Scheme 2.2. The piperic acid was initially activated by p-nitro phenol. All synthesis products showed better results in antibacterial and antifungal activities tests compared to the original structure.



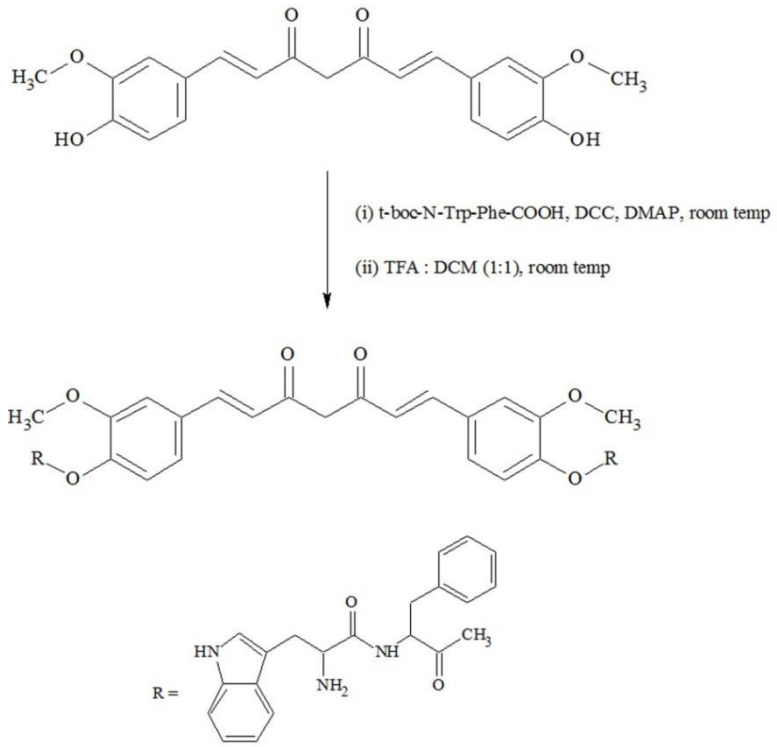
Scheme 2. 1 Synthesis route of 4,4'-di-O-piperoyl–curcumin (Mishra, *et al.*, 2005).



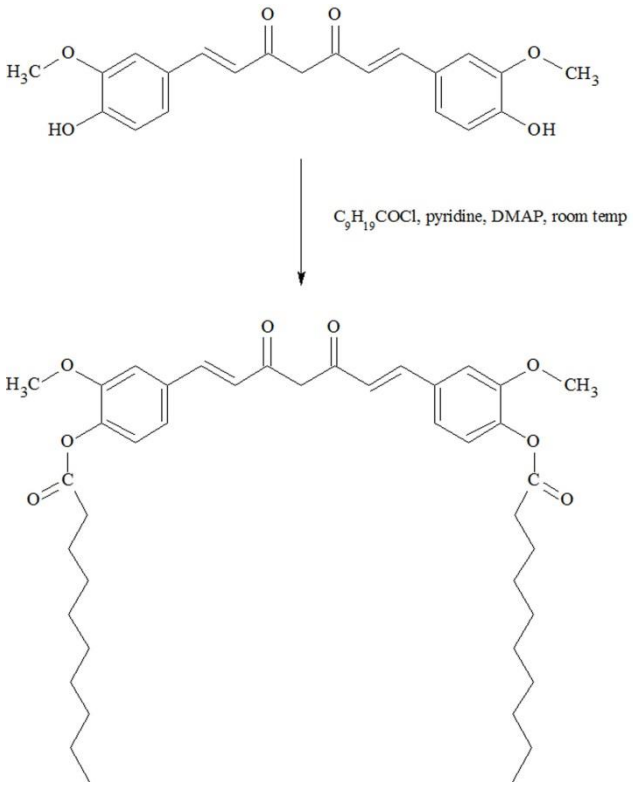
Scheme 2. 2 Synthesis route of 4, 4'-di-O-[glycinoyl-di-N-piperoyl]–curcumin (Mishra, *et al.*, 2005).

Singh, *et al.* (2010) modified curcumin with dipeptide, fatty acids, and folic acid for antibacterial and antiviral activity. Dipeptide selected for the experiment was o-trytophanylphenylalanine with t-Boc protected group as seen in Scheme 2.3. For dipeptide and fatty acids, their reaction with curcumin was esterification which occurred between their carboxyl group and phenolic groups of curcumin as seen in Scheme 2.4. For folic acid, the reaction was more complicated. Folic acid had to be initially activated by p-nitrophenol before reacting with curcumin as seen in Scheme 2.5. Its linkage with curcumin could be either at active methylene group between two ketonic groups or at phenolic groups of curcumin depending on the experimental route chosen. The synthesis route shown in

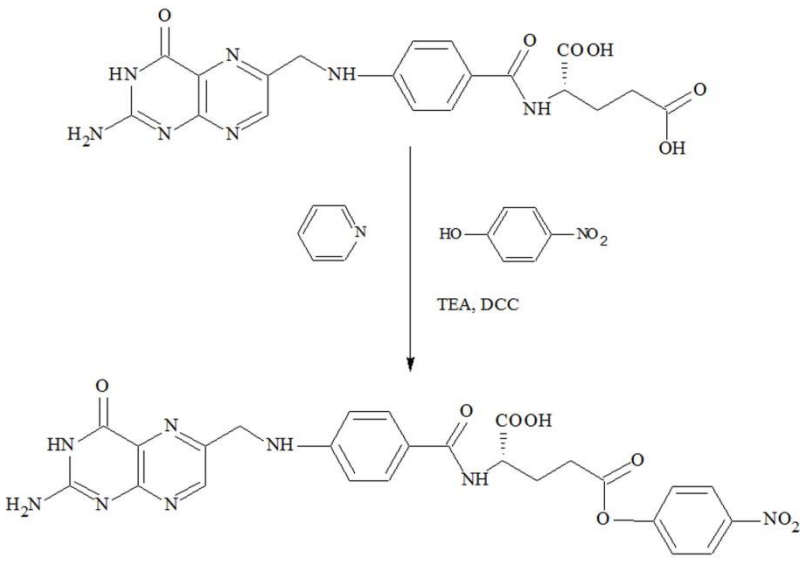
Scheme 2.6 was the reaction of curcumin and activated folic acid at both phenolic groups of curcumin. For another synthesis route, not only activation of folic acid was necessary, but protection of phenolic groups of curcumin with benzoyl chloride was also required. In order to be linked at methylene group, the protected curcumin had to initially react with 2-chloroethanol before reacting with activated folic acid.



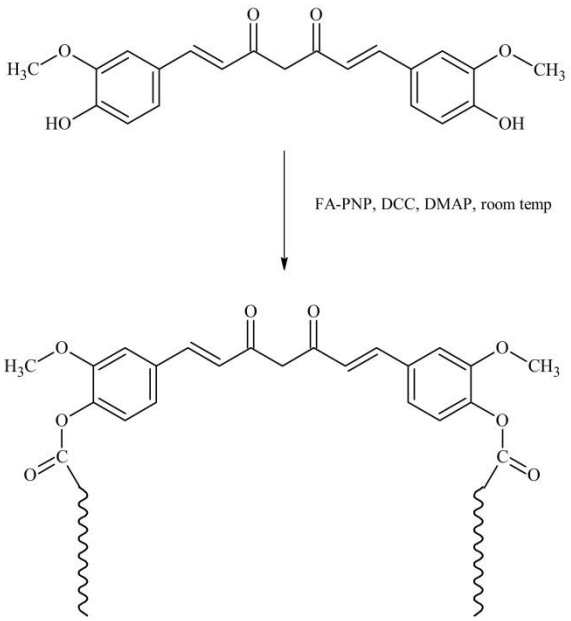
Scheme 2. 3 Synthesis route of di-O-tryphanylphenylalanine curcumin (Singh, *et al.*, 2010).



Scheme 2. 4 Synthesis route of di-O-pamitoyl curcumin (Singh, *et al.*, 2010).

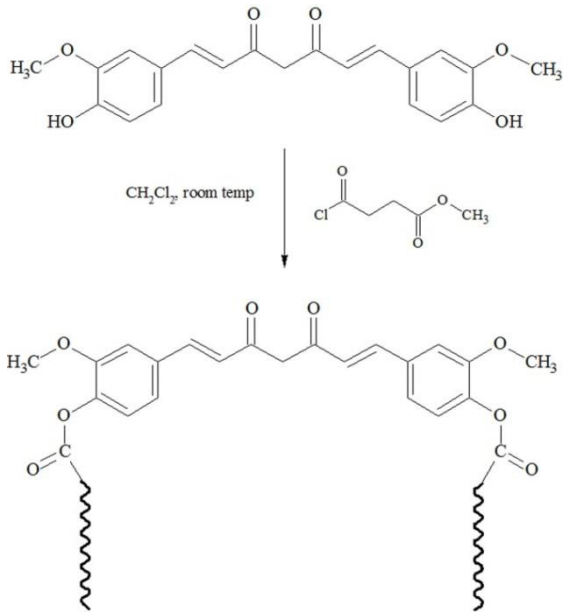


Scheme 2. 5 Synthesis route of p-nitrophenyl ester of folic acid or FA-PNP (Singh, *et al.*, 2010).



Scheme 2. 6 Synthesis route of curcumin bioconjugate at phenolic hydroxyl groups with activated folic acid (FA-PNP) (Singh, *et al.*, 2010).

Wichitnithad, *et al.* (2011) synthesized curcumin from the reaction between acetylacetone and vanillin or (3-methoxy-4-hydroxybenzaldehyde). Then, curcumin was reacted further with succinyl chloride to gain curcumin succinate ester derivative as a prodrug for anti-colon cancer activity as shown in Scheme 2.7.



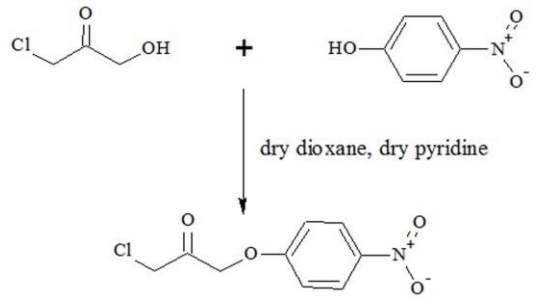
Scheme 2. 7 Synthesis route of curcumin succinate ester

(Wichitnithad, *et al.*, 2011).

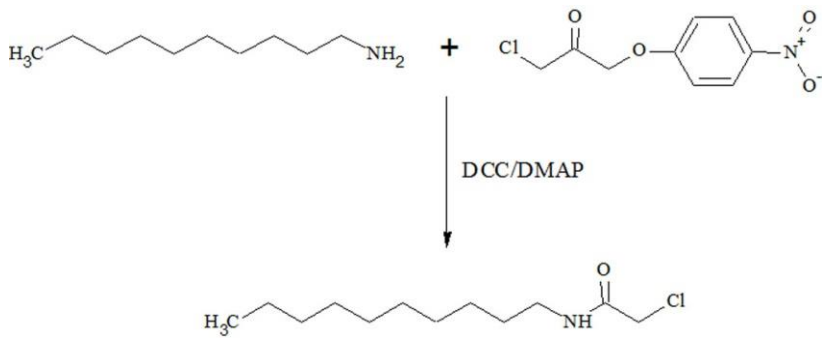
Kapoor, *et al.* (2007) prepared curcumin conjugates containing different internal linkages in the structure via esterification reaction. The reactants used in the experiment with curcumin were amino acids and gallic acid. Acid chloride of amino acids was used, or else these amino acids had to be initially activated by p-nitro phenol. Its amino group was protected by either N-phathaloyl or N-Fomc groups. The reactions between amino acids and curcumin or curcumin derivatives were conducted in dry pyridine at room temperature with the presence of either N,N'-dicyclohexylcarbodiimide (DCC) together with 4-dimethylaminopyridine (DMAP) or hydroxybenzotriazole (HOBt) catalyst. For gallic acid, its phenolic hydroxyl groups were protected by acetylation and its acid chloride was used for the reaction.

Dubey, *et al.* (2008) prepared conjugates of curcumin with N-phthaloyl protected amino acids and piperic acid chloride derivative for medicinal

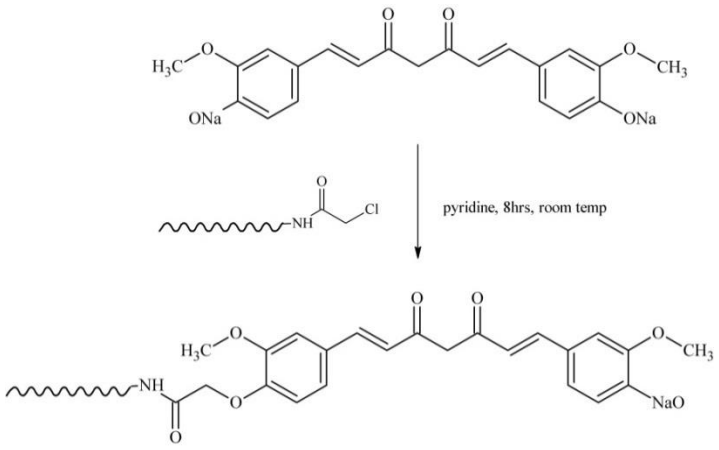
purposes. The solid phase synthesis was used to obtain monoester of curcumin. The resin, solid support, was synthesized from esterification reaction of long chain alky amine with chloroacetic acid initially activated by p-nitrophenol as shown in Scheme 2.8 and 2.9. It was used to react with one phenolic group of curcumin. Thus, there was only one group remaining to react with N-phthaloyl amino acid chloride as seen in Scheme 10 and 11. For curcumin diester, curcumin was directly esterified with amino acid chloride in 1:2 molar proportions.



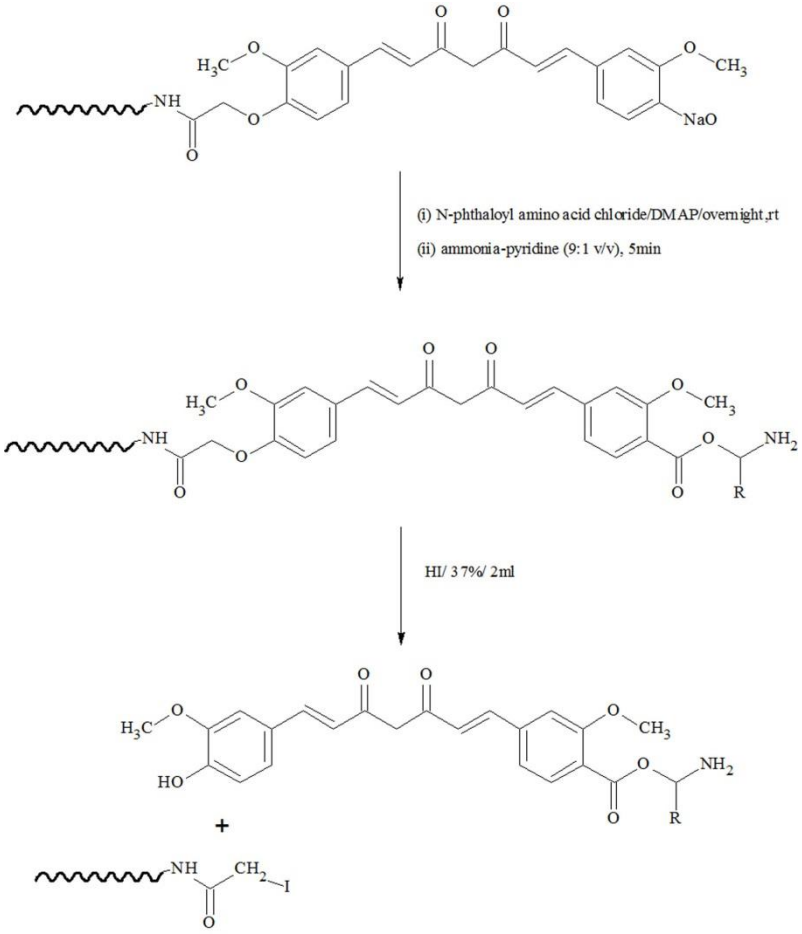
Scheme 2. 8 Activation reaction of chloroacetic acid with p-nitro phenol (Dubey, *et al.*, 2008).



Scheme 2. 9 Reaction of activated ester of chloroacetic acid with long chain alkyl amine on controlled pore glass resin (Dubey, *et al.*, 2008).



Scheme 2. 10 Reaction between a phenolic group of curcumin in alkaline solution with LCAA-CPG (Dubey, *et al.*, 2008)



Scheme 2. 11 Synthesis route of monoester of curcumin (Dubey, *et al.*, 2008).

Parvathy, *et al.* (2010) used t-Boc amino acids to react with curcumin in dry dioxane with the presence of N,N'-dicyclohexylcarbodiimide or DCC as a dehydrating agent, 4–dimethylaminopyridine (4-DMAP) as a catalyst, and triethylamine (TEA) as a base. It was found that when the reaction temperature was maintained between 25-30 ^{o}C, the product yield was higher compared to the reaction at an ambient temperature. Different amino acids yielded different amount of products. The highest yield (76 %) was received from the reaction with alanine whereas the lowest yield (57 %) was from glycine.

The reaction of N-boc-amino acid with curcumin in different organic solvent and other chemical agents also has been reported. For example, Wan, *et al.* (2010) used anhydrous dichloromethane as a solvent media for the reaction and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide or EDCI as a dehydrating agent and DMAP as a catalyst. The reaction was conducted at room temperature under nitrogen atmosphere. Moreover, dipeptide which is a molecule consisted of two amino acids linked by a peptide bond was used to synthesis curcumin bioconjugates. The reaction was conducted in anhydrous dichloromethane with the presence of DCC and DMAP at room temperature for overnight (Singh, *et al*., 2010). To react with curcumin, not only amino acids were used, but amino acid chlorides were involved as well. Dubey, *et al.* (2008) synthesized both monoester and diester of curcumin with N-phthaloyl amino acid chloride in dry pyridine at room temperature for 8 hours.

2.6.2 Modification of Curcumin with Glycidyltrimethylammonium Chloride

Glycidyltrimethylammonium chloride or GTMAC commonly referred to as an epoxide can be synthesized from the reaction of epichlorohydrin with trimethylamine at 25 ^{o}C in an aprotic solvent such as acetone with an excess of epichlorohydrin as solvent (Mcclure, 1970). It is of great interest to modify the chemical structure of curcumin with GTMAC since many findings revealed GTMAC's potential to react with hydroxyl-containing molecules such as polysaccharides and PVA. In most cases, the reaction of GTMAC was conducted in aqueous media which can be of great advantage to the environment. Details of GTMAC are presented hereinafter.

GTMAC can react efficiently with hydroxyl-containing polymers due to its structure. It has two useful functional groups; epoxide and quaternary ammonium groups as shown in Figure 2.6. An epoxide is heterocyclic ether with a three-membered ring which is an equilateral triangle. Accordingly, it has high strain inherent in the structure. GTMAC is, therefore, significantly more reactive to various reagents compared to other cyclic or acyclic ethers. It can react with nucleophiles by an S_N2 reaction at the electrophilic carbons of the C-O bond causing the C-O to break. The epoxide ring is subsequently opened up resulting in the relief of the ring strain. The attack of nucleophiles can occur at either of electrophilic carbons of the epoxide depending on the condition of the reaction. Under alkaline condition, nucleophile will attack unsymmetrical epoxides at the least substituted carbon whereas it will attack the most substituted carbon atom when the reaction condition is acidic (Patrick, 2004).

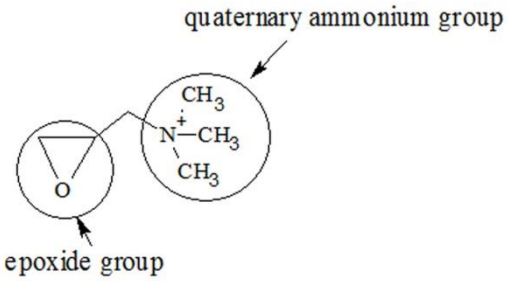


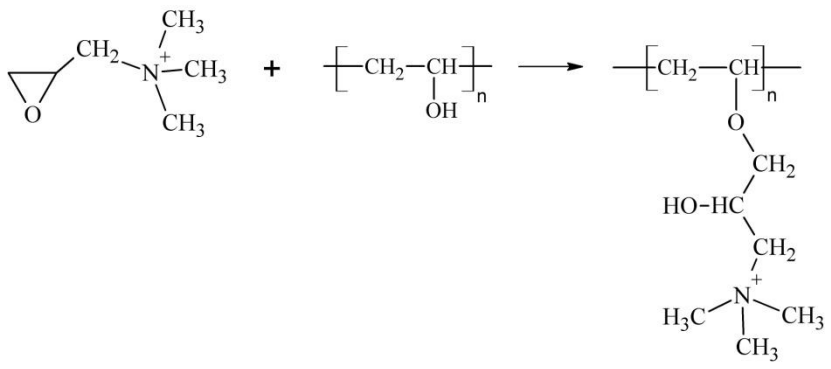
Figure 2. 6 Chemical structure of glycidyltrimethylammonium chloride.

The other functional group in GTMAC is a quaternary ammonium group. This functional group has a permanent positive charge at nitrogen atom attached to four organic groups. Quaternary ammonium compounds have been used to incorporate into certain substances for water soluble property (Liu, *et al.*, 1998; Staneva, *et al.*, 2015; Xiao, *et al.*, 2002). The quaternary ammonium groups imparted to structure of a compound can increase not only water solubility of the material, but antibacterial activity could be enhanced as well. Therefore, the epoxide group of GTMAC was used to react with phenolic group of curcumin and the quaternary ammonium moiety of a novel structure of curcumin was expected to assist water solubility capability of curcumin.

For compounds containing hydroxyl groups in their backbone structures, a great deal of research works have been published. These substances include both natural and synthetic compounds. Synthetic materials are mainly PVA. Natural compounds cover tannin, dextrin saccharides, chitin, and certain polysaccharides such as hemicellulose and hydroethylcellulose.

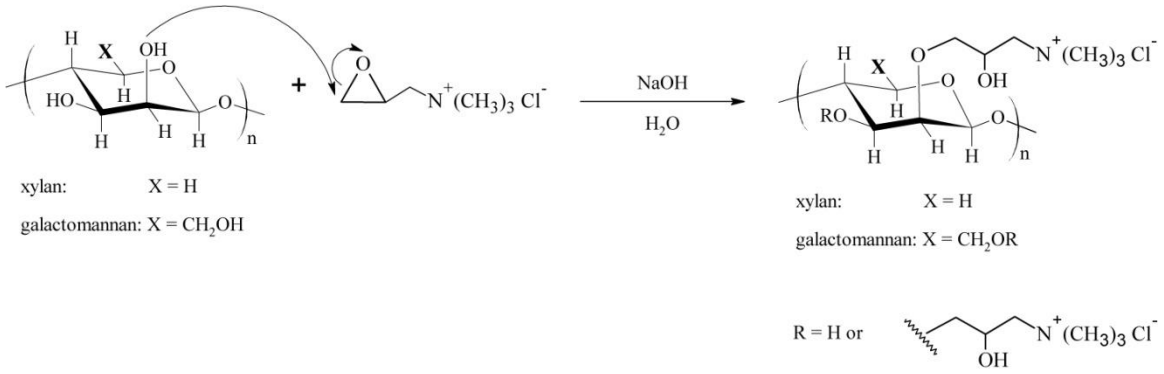
PVA was modified with GTMAC to be a cationic dry-strength additive for non-wood based papermaking application. When non-wood raw materials are used in

papermaking, the mechanical properties of papers are affected due to high silica content of the materials. Hence, additives with excellent adhesion property to cellulose are necessary to increase the number of hydrogen bonding between fibers. Their binding strength can be increased as a result. One of the most dominant polymers involving in several applications in papermaking processes is PVA. It has hydroxyl groups in every repeating unit. When PVA dissociates, it gains negative charges. The electrostatic repulsion between its negative charges and those of hydroxyl groups of cellulose can occur resulting in hindering the interaction of PVA with cellulose. Accordingly, positively charged modified PVA or a mixture between PVA and a crosslinking agent have been used as a new approach to provide a better interaction with cellulosic fibers. Fatehi, *et al.* (2009) prepared cationic-modified PVA by heating the solution mixture of PVA and GTMAC in alkaline aqueous media at 80 ^{o}C for 1 hour. The analysis results showed that PVA was mainly modified with GTMAC at the hydroxyl groups on its backbone. The cationic modified PVA could function well as a dry strength additive. It could increase fiber bonding to a large extent. The scheme of reaction is shown in Scheme 2.12.



Scheme 2. 12 Reaction of GTMAC with PVA ((Fatehi, *et al.*, 2009)).

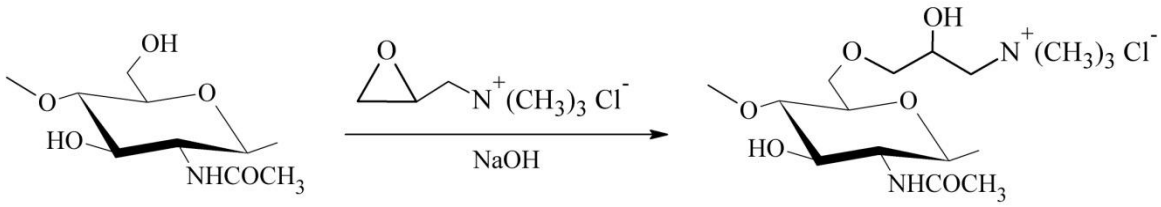
Bigand, *et al.* (2011) successfully grafted two hemicellulose polymers with GTMAC in alkaline aqueous media at the temperature of 60 ^{o}C through etherification reaction. They were galactomannan and xylan. Both of them are polysaccharides but with different average number of hydroxyl groups per sugar unit. The scheme of the reaction is shown in Scheme 2.13.



Scheme 2. 13 Etherification reaction of xylan and galactomannan with GTMAC in sodium hydroxide aqueous solution (Bigand, *et al.*, 2011).

In this reaction, the concentration of hemicellulose and GTMAC influenced the degree of substitution of hydroxyl groups and product yields. Another interesting reaction between a hydroxyl-containing compound with GTMAC is the cationization of dextrin, a low molecular weight carbohydrate, in alkaline aqueous solution. The reaction was carried out at low temperature only 25 ^{o}C. In this work, continuous stirring apparatus and ultra-high pressure were used to assist the reaction and their reactivities were compared by means of degree of substitution (DS). The result suggested that using high pressure could shorten the reaction time from 5 hours to only 30 minutes to achieve similar DS values (Cho, *et al.*, 2013).

The reaction of chitin and GTMAC is another example which the substitution reaction occurred at hydroxyl groups of chitin. Chitin which is a polysaccharide of 2-acetamido-2-deoxy-D-glucose has 2 molecular organizations: α- and β-forms. β-chitin has comparatively looser structure. Therefore, it can be swollen by small molecules such as water, ethanol and 2-propanol. Chen, *et al.* (2010) prepared a high molecular weight quaternized chitin derivative directly from β-chitin. It was dispersed in 2-propanol and subsequently reacted with GTMAC under alkaline condition at 40 ^{o}C for 6 hours. Due to the acetamido group (-$NHCOCH_3$), no amine group is available to react with other reagents. In β-chitin, the quaternization reaction occurred only at the C-6 hydroxyl group. The proposed scheme of reaction was shown in Scheme 2.14.

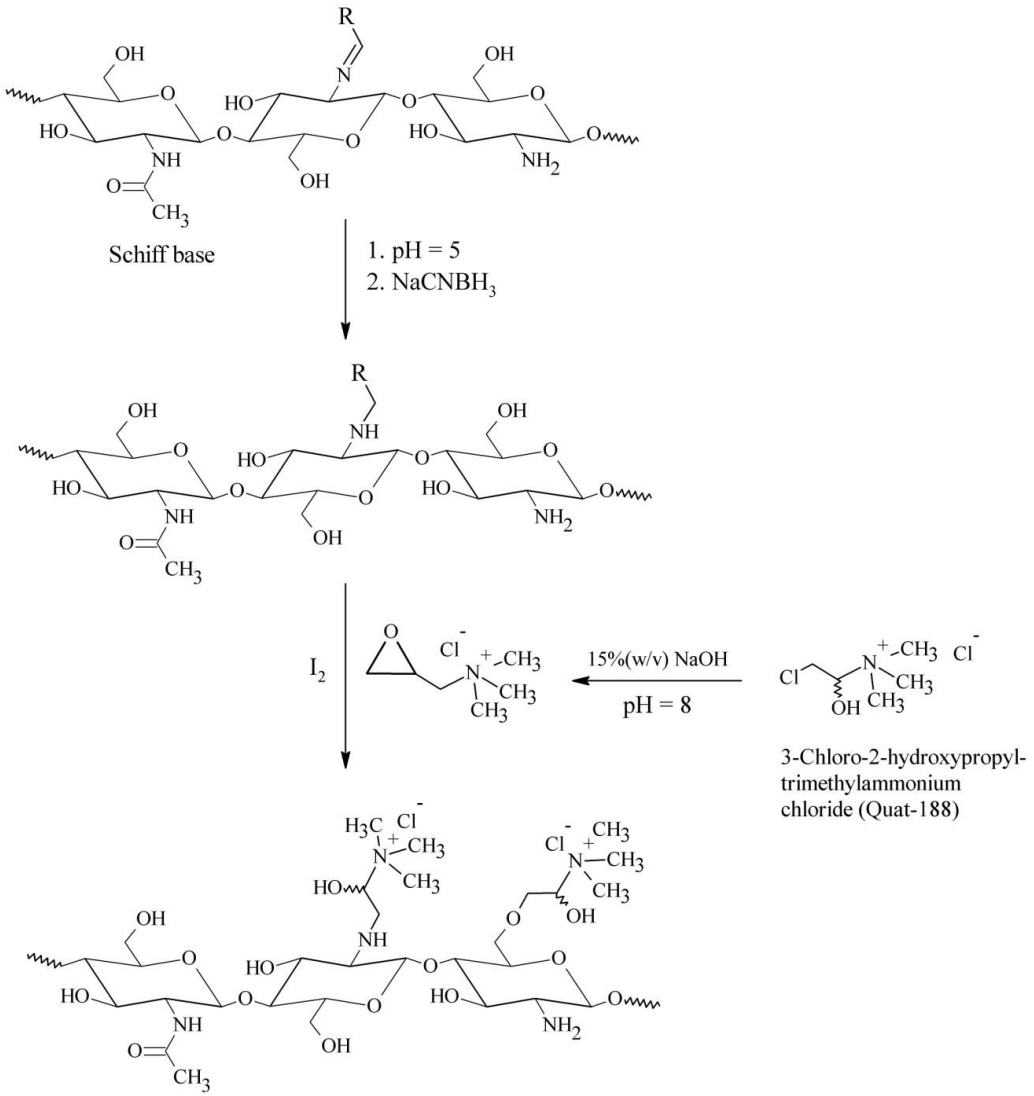


Scheme 2. 14 Synthesis route of quaternized β-chitin (Chen, *et al.*, 2010).

GTMAC is very active to react with not only hydroxyl group-containing molecules but also amino-bearing macromolecules including polysaccharides such as hemicellulose, pectin, lignin and chitosan. From the reaction of polysaccharides with GTMAC, cationic derivatives with a variety of functions and properties have been produced and widely used in diverse areas such as biomedical field as drug or protein delivery (Chaleawlert-umpon, *et al.*, 2011; Cui, *et al.*, 2010; Shi, *et al.*, 2011; Wu, *et al.*, 2006; Yu, *et al.*, 2011), water and wastewater treatment (Shimizu, *et al.*, 2005; Spinelli, *et al.*, (2004), cosmetics

and external disinfection (Chi, *et al.*, 2007) and antibacterial agents (Nam, *et al.*, 2001; Sajomsang, *et al.*, 2009).

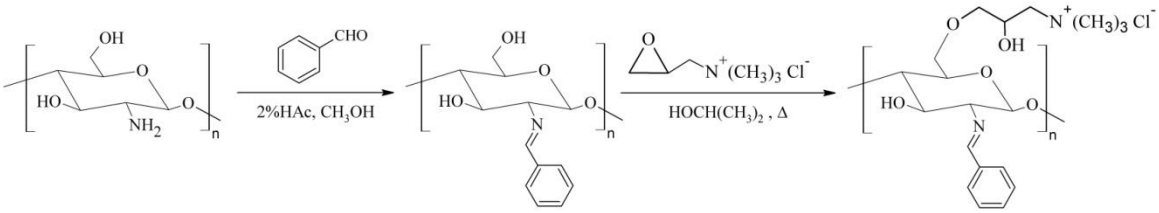
Chitosan is one of the polysaccharides capable of reacting with GTMAC at all reaction conditions whether it is acidic (Chi, *et al.*, 2007; Cui, *et al.*, 2010; Shimizu, *et al.*, 2005), alkaline (Chen, *et al.*, 2010; Sajomsang, *et al.*, 2009; Yu, *et al.*, 2011) or neutral (Nam, *et al.*, 2001; Spinelli, *et al.*, 2004; Wu, *et al.*, 2006). Chitosan has both amino and hydroxyl functional groups in its backbone and both groups can act as reactive nucleophiles to attack either carbon atom at C-O-C unit of GTMAC. The substitution reaction reported in the literature predominantly occurs at amino groups at all conditions since amino groups had much stronger nucleophilicity to open epoxide ring of GTMAC than hydroxyl groups (Lim & Hudson, 2004). Nevertheless, the substitution reaction occurred at hydroxyl groups of chitosan also has been reported. Sajomsang, *et al.* (2009) synthesized quaternary ammonium chitosan and its derivatives using commercial N-(3-chloro-2-hydroxypropyltrimethylammonium chloride) or Quat-188. Chitosan and its derivatives were regenerated before reacting with Quat-188, which was converted to be an epoxide under alkaline condition. It was then became a quaternizing agent to modify both amino and hydroxyl groups of regenerated chitosan and its derivatives. However, the substitution occurred mainly at the primary amino groups. The scheme of the synthesis method is shown in Scheme 2.15.



Scheme 2. 15 Etherification reaction of mono and disaccharide chitosan derivatives, synthesized via Schiff base intermediates, with commercial quaternary ammonium epoxide under alkaline condition (Sajomsang, *et al.*, 2009).

Another work related to the hydroxyl substitution reaction is the reaction of benzaldehyde-modified chitosan with GTMAC. The reaction took place at hydroxyl group, since amino groups were no longer available. They were reacted

with benzaldehyde to form chitosan Schiff base as shown in Scheme below (Fu, *et al.*, 2011).



Scheme 2. 16 Synthesis route of benzaldehyde-modified chitosan with GTMAC (Fu, *et al.*, 2011).

GTMAC can undergo a ring opening reaction in alkaline condition usually through a S_N2 mechanism with compounds bearing hydroxyl groups. The reaction is mainly influenced by certain parameters as follows: molar ratio of GTMAC to other reactants, type and concentration of alkaline, media of reaction, reaction temperature, and time.

The Effect of GTMAC to Reactant Molar Ratio

Under a given condition, the rate of reaction usually increases with an increase in concentration of reactants. For a reaction with hydroxyl-bearing compound, an increase in molar ratio of GTMAC to hydroxyl-bearing compound also increases the rate of reaction and % product yield. The optimum concentration of GTMAC depends on the availability of reaction sites of hydroxyl-bearing compound (Fatehi, *et al.*, 2011). The excess amount of GTAMC has no significant effect on the rate of reaction (Wang & Xie, 2010).

The Effect of Type and Concentration of Alkaline

Alkaline is important for the reaction of GTMAC with hydroxyl-bearing compounds. It works as a catalyst to activate both hydroxyl groups of the compound into nucleophilic hydroxide and the epoxide ring of GTMAC to open into the corresponding diol as illustrated in Figure 2.7 (Bigand, *et al.*, 2011).

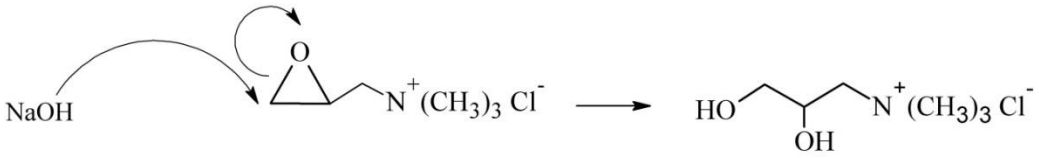


Figure 2. 7 Ring opening of epoxide into the corresponding diol (Bigand, *et al.*, 2011).

Without alkaline, no reaction occurred as observed from the reaction of xylan with GTMAC conducted by Bigand, *et al.* (2011). Rate and efficiency of the reaction and the degree of substitution (DS) increased with an increase in alkaline concentration until the maximum amount was reached. The excess then showed negative impact on the reaction such as lower the DS of the reaction between GTMAC and xylan. A plausible reason for this phenomenon was possibly derived from the faster rate of ring opening of the epoxide than the rate of substitution of GTMAC at hydroxyl functional groups of xylan. As alkaline concentration increased, the degradation of the epoxide was predominant and competed with less reactive hydroxyl groups. The efficiency of the reaction was then lower. Therefore, concentration of alkaline used in the reaction has to be optimized to maximize the activation of hydroxyl groups but minimize the formation of hydrolysis byproduct (Bigand, *et al.*, 2011).

Type of alkaline is also important to the reaction since the influence of individual alkalines to the rate of hydrolysis is different. The order from low to high impact on hydrolysis rate of epoxide group is as follows: CH_3COONa < NH_4Cl < Na_2CO_3 < NaOH (Bendoraitiene, *et al.*, 2006). Moreover, strong alkaline can also hydrolyze the modified products such as cationic-modified PVA (Fatehi, *et al.*, 2011) and cationic hemicellulose (Ren, *et al.*, 2007).

The Effect of Media of Reaction

Reaction media obviously has an influence to the reaction efficiency (Ren, *et al.*, 2007). For the reaction of GTMAC with hydroxyl or amino-containing reactants, water has been commonly used. It is an environmental friendly solvent. However, it can break a bond in a molecule with no exemption to the molecule of GTMAC. Very low dilution of GTMAC solution could also lead to a fivefold increase in the rate of hydrolysis of its epoxy groups (Bendoraitiene, *et al.*, 2006) as detected from the reaction of GTMAC with PVA conducted by (Fatehi, *et al.*, 2011).

The Effect of Reaction Temperature

Increasing reaction temperature is normally an effective way to accelerate the rate of reaction. However, at high temperature, side reaction may occur. The optimum temperature should be the temperature at which the reaction is best carried out with less side reaction (Kavaliauskaite, *et al.*, 2008).

The Effect of Reaction Time

At a constant temperature, product yield usually increases with reaction time until it reaches a maximum. Prolonged reaction time, in certain cases, can affect

the reaction efficiency or the stability of reaction products. For example, the hydrolysis of cationic starch (Wang & Xie, 2010) and cationic PVA *(*Fatehi*, et al.*, 2011) was higher as the reaction time was extended.

2.7 The Key Issues/Problems of Research

Over a few decades, the demand for green or eco-friendly textile products has been greatly increased. A number of scientific studies on the development of dyes, chemicals, and suitable application procedures have been conducted to achieve these particular requirements (Samanta, *et al.*, 2014). Natural dyes also have been focused on as a potential alternative to synthetic dyes. Since natural dyes have certain limitation/disadvantages such as low color yield and poor fastness properties, various approaches have been conducted to develop techniques or apply technologies applicable to production and application of natural dyes.

For curcumin, the improvement of its dyeing quality has been diversely carried out starting from the extraction of curcumin from turmeric raw material until the optimization of its dyeing conditions. Energy surface modification technologies such as plasma treatment, gamma radiation, and UV radiation were also introduced to investigate their influence to color yield in textile coloration applications. They were applied to both turmeric before extracted and textile substrates, i.e., polyester and cotton to be dyed.

In curcumin dyeing, the optimization of dyeing conditions was attempted both with and without the use of mordants. A variety of mordants including biomordants were studied for their capability to intensify color in dyeing and to

improve color fastness properties as well as to enhance antibacterial properties on dyed substrates.

From literature study related to curcumin, it was found that chemical modification of curcumin structure was conducted mainly using carboxyl-containing compounds, such as amino acids and gallic acid, to improve its potential use in food and medicinal areas. However, no studies and research in regard to chemical modification of curcumin or other natural dyes was engaged in textile coloration applications.

Moreover, another reagent containing quaternary ammonium group was found successfully used in chemically modifying hydroxyl-bearing macromolecules such as polyvinyl alcohol and chitosan to enhance their functions and properties. It is glycidyltrimethylammonium chloride and its reaction is comparatively simple and environmentally friendly. Importantly, no research work has been reported using this reagent to modify the structure of curcumin.

Therefore, an amino acid and glycidyltrimethylammonium chloride were selected to modify the chemical structure of curcumin to improve its dyeability and other functional properties, i.e., antibacterial and UV protection properties.

Chapter 3: Research Methodology

3.1 Introduction

This experimental work is divided into 3 parts as follows:

(1) The extraction of volatile oil and curcumin natural dye from *Curcuma longa L.*and dyeing applications of curcumin on synthetic and natural fibers;

(2) The improvement of dyeability, dyeing properties, and other properties of curcumin natural dye mainly towards cotton with the ecologically friendly chemical modification using N-protected amino acid and quaternary ammonium epoxide; and

(3) The study of the dyeability and other properties of textiles dyed by curcumin derivatives.

The first part focused on the full usage of natural raw material of curcumin. The experimental route started from extraction of volatile oil from turmeric powder by steam distillation method. Then the steam distillation crude extract was isolated to obtain curcumin using ethanol solvent. The dyeing applications of curcumin on a variety of fibers were subsequently studied.

The second part was associated with the chemical modification of curcumin using two different approaches and reagents. The first approach was carried out via nucleophilic addition/elimination reaction with N-protected glycine, whereas the latter approach dealt with glycidyltrimethylammonium chloride via nucleophilic substitution reaction.

The final part principally involved the applications of chemically modified curcumin. Standard test methods and procedures for properties and functions of curcumin products were conducted.

In this chapter, materials and chemicals used for all experiments were presented. Important characterization instruments and machines including standard test methods related to all applications are elaborated hereinafter.

3.2 Materials and Chemicals

3.2.1 Textile Substrates

The pretreated woven cotton fabric (122 g/m^2) and 100 % acrylic knitting yarn (bulky) (No.20/3) was kindly supplied by local textile mills in Thailand. Polyester fabric (156 g/m^2) and poly(lactic acid) or PLA fabric (118 g/m^2) were purchased from China Dyeing Holdings Ltd.

3.2.2 Chemicals and Reagents

80 mesh sieve of turmeric powder with 7.49 % moisture was obtained from Thailand. 98 % Curcumin CAS No, 458-37-7 was purchased from Acros Organics, Belgium. 99 % N-phthaloylglycine were supplied by Fluka, Switzerland. Glycidyltrimethylammonium chloride was supplied from Aldrich USA. Other chemicals and organic solvents used were all reagent grades purchasing from local suppliers and they were used without any further purification. These chemicals and solvents were dicyclohexylcarbodiimide (DCC), 4-dimethylaminopyridine (4-DMAP), sodium hypophosphite, sodium carbonate anhydrous, formic acid, citric acid, dichloromethane,

dimethylformamide, ethyl acetate, 1,2-dichloroethane, chloroform, N-hexane and absolute ethanol.

3.3 Instrumentation and Machines

Analytical instruments are powerful tools for the characterization of the structure and composition of a compound. In this work, several analytical instruments were used. They were gas chromatography-mass spectrometry (GC-MS), Fourier transform-infrared spectrometry (FT-IR), Nuclear magnetic resonance spectroscopy (NMR), liquid chromatography-mass spectrometry (LC-MS). UV-visible spectroscopy was also employed for both qualitative and quantitative determination of the compounds related.

3.3.1 Gas Chromatography – Mass Spectrometry (GC-MS)

GC-MS is one of the hyphenated analytical techniques. It comprises of a gas chromatography coupled to a mass spectrometry. It is one of the most sensitive and selective analytical methods for the separation, identification, and quantification of individual components of complex organic mixtures. GC-MS technique offers finer degree of identification of analyte. GC assists the differentiation of components of similar fragmentation patterns while MS aids the differentiation of components of similar or same retention time from GC and functions as a detector.

GC-MS essentially consists of 3 components: a gas chromatograph, a mass spectrometer, and a data system. It is limited to analyze only volatile organic compounds which are stable in high temperature in GC conditions. A block diagram of a typical GC-MS system is shown in Figure 3.1.

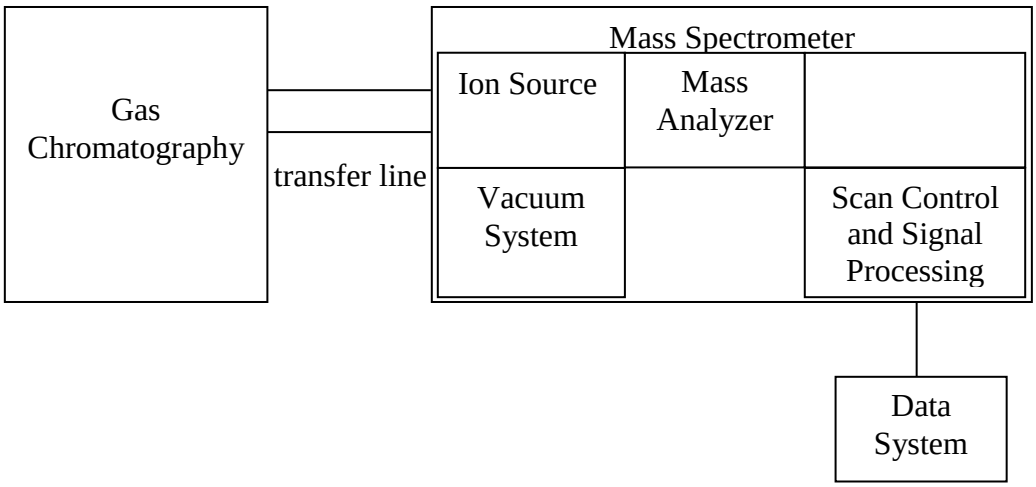


Figure 3. 1 Block diagram of typical GC-MS system (Handley & Adlard, 2001).

In this work, GC-MS was selected to analyze components of extracted product from steam distillation of turmeric powder. Charged particles were analyzed after the volatile oil was injected into an airtight chamber of GC and mass to charge ratio of individual molecules was measured at the mass spectrometer unit.

In general, individual components in a mixture may have different chemical and physical properties. The relative affinity of each component for stationary phase and mobile phase should be different. Components move through the column at different speed and, therefore, they can be separated. The analyte molecules separated in GC column are ionized in mass spectrometer prior to analysis. The detector unit creates an electronic signal when the component is detected. This signal is then converted to be a graph called chromatogram. Each peak in chromatogram represents a signal of the fragmented component eluted from GC column into the detector (MS). The x-axis is retention time, a time from when injection is made to when elution occurs, and the y-axis is the signal intensity. Mass spectrum retrieved from individual peaks in chromatogram allows

identification of components by comparing with standard reference libraries (Handley & Adlard, 2001; Patel, *et al.*, 2010).

In this work, Hewlett-Packard GC 6890 series coupled to the Agilent Technologies 5973 MSD system was selected to analyze components of extract product from steam distillation of turmeric powder. The extract product was diluted with absolute ethanol and injected onto one end of the gas chromatographic column with the splitless technique. The mixture was immediately vaporized and moved through the column by purge of Helium gas as a carrier. The condition of the analysis was as follows: the flow rate of Helium carrier gas was 1.7 ml/min; injection temperature was 240 ^{o}C; detector temperature was 230 ^{o}C; oven temperature was programmed at 70 ^{o}C for 5 min and then increased to 230 ^{o}C at the rate of 5 ^{o}C/min and held constant for 5 min.

3.3.2 Fourier Transform Infrared Spectroscopy (FT-IR)

Fourier transform infrared spectroscopy or FT-IR is a method of obtaining infrared absorption spectra by first measuring the interferogram of a sample signal using an interferometer, and then performing a Fourier Transform on the interferogram to obtain the spectrum (Smith, 2011). It is particularly useful in characterizing molecular bonds and functional groups by measuring the vibrational frequencies of atoms within a molecule.

When exposed to infrared radiation, molecules selectively absorb energy in the form of photon at specific wavelengths and undergo transitions between vibrational energy levels. An increase in vibrational energy causes atoms in the molecules to be stretched far apart from one another. The average bond length of the molecule increases. Atom bending angle can be changed. The wavelengths

that are absorbed by the molecule are characteristics of the molecular structure. A molecule can only absorb certain photons to change its vibrational energy state.

Absorption interaction between the incident photons and sample atoms in the molecules are recorded and intensity of signal is plotted against the optical path difference to be interferogram. Fourier transformation is used to convert the interferogram to final IR spectrum with relative absorbance (or transmittance) against the incident photon frequency (wavenumber) (*The UC Davis ChemWiki-infrared spectroscopy.*2015; Rehman, *et al.*, 2012).

Perkin Elmer Spectrum 100 was used to obtain FTIR spectra of curcumin and its derivatives. The preparation methods used for commercial curcumin and extracted curcumin were different since they were available in different forms. The commercial curcumin was in the form of a dry powder, whereas the extracted curcumin was similar to a sticky, gum-like substance. The samples for FT-IR were prepared as described below before being scanned on the wavenumber (ν) between 4000 and 450 cm^{-1}.

For the commercial curcumin, its powder was mixed and milled homogenously with potassium bromide and then compressed into a thin pellet using KBr press. It was subsequently used to analyze for an infrared absorption spectrum.

For the extracted curcumin, it was dissolved in dichloromethane and the solution was then dropped onto a dry KBr circular plate. A thin film of extracted curcumin was formed on the plate, after dichloromethane was evaporated. The film was then analyzed by FT-IR.

Furthermore, Perkin Elmer Spectrum 100 was also used to provide spectra for untreated and the modified curcumin-treated cotton fabrics by equipping with a universal attenuated total reflectance accessory. The FT-IR spectra recorded were the average of 32 individual scans at 4 cm^{-1} spectral resolution in the 4000-600 cm^{-1} range. Types of molecular bonds and functional groups of fabrics were analyzed.

3.3.3 UV-Visible Spectrophotometer

A UV-visible spectrophotometer is an instrument which measures reflectance or absorbance characteristics of a sample (Martin & Pretzel, 1991). Ultraviolet (UV) radiation has wavelength range of 200-400 nm and visible (VIS) light has wavelength range of 400-800 nm. When exposed to UV-visible light, a sample molecule absorbs energy from the radiation. The energy is sufficient to cause electronic transitions within the molecule. Its electrons are excited to a higher energy molecular orbital, giving rise to an absorbance at a wavelength specific to individual molecules. The absorbance of light by the sample molecule can be measured and plotted against the wavelengths of the incident radiation to provide a UV-VIS spectrum. The concentration of a given sample can be determined using Lambert-Beer Law (Reusch, 2013).

Two different models of spectrophotometer were used throughout this work, namely, Perkin-Elmer Lambda 18 and Varian Cary 300 Conc UV-Visible Spectrophotometer. The first type was used to record UV-visible spectra of the solution of curcumin and its derivatives, whereas the latter was used to measure ultraviolet protection factor (UPF) of the fabric samples. Experimental procedure

of Varian Cary 300 Conc UV-Visible Spectrophotometer for UPF measurement is described as follows.

All samples were measured at 4 different directions for the rated ultraviolet protection factor with a Varian Cary 300 Conc UV-Visible Spectrophotometer. Sample transmission data (% *T*) were recorded in the range of 280-400 nm. Mean of the ultraviolet protection factor (UPF) was calculated according to the method described in the Australian/New Zealand Standard AS/NZS 4399:1996.

3.3.4 Liquid Chromatography-Tandem Mass Spectroscopy (LC-MSMS)

Liquid Chromatography-Tandem Mass Spectroscopy (LC-MSMS) is a combination of liquid chromatography system with tandem mass spectrometers. Main function of LC is to separate sample components based on the difference in polarity. Eluting substances from LC are subsequently introduced into the mass spectrometer where charged ions are generated and subsequently analyzed for their mass-to-charge ratio. Structures of precursor molecules are identified from their molecular ions (Li, *et al.*, 2013). In this work, Waters® Micromass® Q-Tof 2 quadrupole time-of-flight mass spectrometer (LC-MSMS) equipped with electrospray ionization was used to obtain mass spectra of the modified curcumin.

3.3.5 Nuclear Magnetic Resonance (NMR)

Nuclear Magnetic Resonance or NMR is a technique widely used for determining the structure of molecules in solution (Jacobsen, 2007; Roberts & Lian, 2011). When placing a sample in a homogeneous magnetic field and applying suitable frequencies of electromagnetic energy, an NMR spectrum is

obtained (Becker, 1980). To extract the structural information from the NMR spectrum, three basic following parameters are important: the number of signals in the spectrum, the relative signal intensities, and NMR chemical shifts (δ) (Leardi, 2003). Chemical shifts are values usually measured by reference to a standard compound and being independent of the magnetic field strength of the NMR spectrometer (Cavanagh, *et al.*, 2007).

NMR is a spectroscopic technique related to the magnetic properties of certain atomic nuclei of molecules. A molecule may consist of a single atom or a group of atoms held together by valence forces. An atom consists of a heavy nucleus surrounded by light electrons. All nuclei carry a positive charge but only certain have a charge spin on the nucleus axis causing a magnetic dipole along the axis. Therefore, it has an angular momentum described in term of spin quantum number.

An atomic nucleus with either an odd atomic number or odd atomic mass has a nuclear spin property. Since nuclei contain positive charges from protons, their rotation created a current loop, causes a magnetic field line. Each spinning direction is random. There is no energy difference between the spin states in any one direction.

As an atom is placed in a strong external magnetic field, the spinning nucleus can align either in the same direction of the applied magnetic field or in the opposite direction depending on the energy state of its magnetic moment.

In NMR application, the radio frequency electromagnetic radiation is applied to induce the transition of nuclei from lower state to higher state since nuclei

absorb certain energy. When the radio frequency signal is switched off or the energy is removed, the spinning nuclei return to their thermodynamically stable states. The energy absorbed when a transition from lower energy level to a high energy level occurs is released. As the excited nuclei return to their lower energy level, the measurable radio signals re-emit at the frequency of the splitting as the NMR signal. The difference in observed resonance frequencies of nuclei relative to the resonance frequency of a standard, usually tetramethylsilane or TMS, is defined as the chemical shifts. (Biewer, 2015).

In this work, a Bruker Ultrashield Advance Pro 400 MHz NMR spectrometer was performed using $CDCl_3$ as a solvent and tetramethylsilane as internal standard to obtain ^1H spectra for curcumin and its derivative.

3.3.6 Reflectance Spectrophotometer (Macbeth CE-7000A spectrophotometer)

The color measurement was performed by Macbeth CE-7000A spectrophotometer. After stored in a conditioning room for 24 hours, all treated fabrics were measured for their spectral reflectance data at the wavelength between 400-700 nm with the Scope System (from Gain Associate Inc.) using Macbeth CE-7000A spectrophotometer. The machine was set up for essential features as follows: large area aperture, UV filter included for cotton and PLA fabrics and excluded for polyester fabrics and acrylic yarns, and specular component included under illuminant D65 and 10^o observer.

3.4 Standard Test methods

3.4.1 Antibacterial Finishes on Textile Materials: AATCC 100-2012

The antibacterial activities of the cotton samples treated with curcumin derivative were quantitatively evaluated against Escherichia coli (*E.coli*) according to AATCC 100-2010 test method. The test fabric and control fabric were cut to make circular swatches with a diameter of 4.8 ± 0.1 cm and all swatches were then sterilized in the autoclave at 121 ^{o}C for 15 min before conducting the test.

All swatches were placed separately in sterile Petri dishes and inoculated evenly with a nutrient broth culture containing 10^5-10^6 colony-forming units (CFU) of bacteria/ml. Individual inoculated swatches were then transferred aseptically to a wide-mouth glass jar with a lid that can be closed tightly to prevent evaporation. The neutralizing solution was added into each of the jars containing the inoculated test samples, the inoculated control samples and uninoculated test samples. Afterward, the jars were shaken vigorously for 1 minute before making the serial dilutions. Each aliquot of the diluted bacterial solution was placed on a nutrient agar plate and incubated at 37 ± 2 ^{o}C for 48 h. The number of viable colonies of the bacteria on the agar plate at "0" contact time was finally counted.

The same procedure was applied to obtain the number of bacteria colonies over the desired contact period except that the jars containing sample swatches had to incubate at 37 ± 2 ^{o}C for 24 h before adding the neutralizing solution. After counting the number of viable bacteria colonies of both contact times on the agar plates, percent reduction of bacteria (R%) was calculated using the following equation.

$$R\ (\%) = (A-B)/A \times 100$$

Where A and B are the number of bacterial colonies in inoculated test sample or control sample after inoculation (at "0" contact time) and after incubation over the desired contact time, respectively.

3.4.2 Standard Test Method for Determining the Antimicrobial Activity of Antimicrobial Agents under Dynamic Contact Conditions: ASTM E2149

The antibacterial properties of cotton treated with curcumin derivative were quantitatively evaluated against *S. aureus* according to ASTM E2149. The test specimens were placed in an individual flask containing working dilution of *S. aureus* for a desired contact time.

The nutrient broth culture of *S. aureus* was prepared and then diluted with the sterile buffer solution to give a final concentration of 2.6×10^5CFU/ml. 50 ml of the working dilution of *S. aureus* was transferred to all sterilized flasks to which one piece of sterilized treated and untreated specimens weighing 1.0 ± 0.1 gram was individually added, including "inoculum only" flask. All flasks were screwed and they were shaken at the maximum stroke for 5 hours. Then the serial dilutions were carried out and were plated onto nutrient agar in Petri dishes. All the Petri dishes were incubated at 35 ± 2 ^oC for 24 hours. The number of colonies in the Petri dishes was enumerated. The percent reduction was then calculated using the following formula.

$$\text{Reduction, \% (CFU/ml)} = (B-A)/B \times 100$$

Where:

A= CFU per milliliter for the flask containing the treated substrate after 5 hours contact time, and

B = CFU per milliliter for the flask containing the untreated substrate or the inoculums only flask after 5 hours contact time.

3.4.3 Sun Protective Clothing-Evaluation and Classification: Australian/New Zealand Standard AS/NZS 4399:1996

All specimens were individually tested and rated for ultraviolet protection factor by measuring 4 times in different directions with a Varian Cary 300 Conc UV-Visible Spectrophotometer. Transmission data (%*T*) of each specimen were recorded in the range of 280-400 nm. Mean of the ultraviolet protection factor (UPF) was calculated from spectrophotometric transmission data according to the Australian/New Zealand Standard AS/NZS 4399:1996.

3.4.4 Colorfastness to Laundering: Accelerated-AATCC Test Method 61-2010 test No. 2A

AATCC Test Method 61-2010 was selected to evaluate mainly 2 properties: color fastness to laundering and durability of UV protection properties after repeated laundering. This test method simulates home or commercial laundering with five different washing conditions. In this work, the test condition No. 2A was specifically selected. Wash testing was conducted under accelerated conditions, simulating five home machine launderings using medium or warm setting at the temperature in the range of 38 ± 3 ^{o}C. Color change and stain of dyed substrates were evaluated in color fastness test.

For color fastness test, the specimens with the size of 50 x 150 mm were prepared and attached along one 50 mm edge to multifiber test fabric by positioning the stripe of wool on the right side. All fiber stripes were arranged

parallel to the length of the specimens. For durability of UV protection properties against repeated laundering, the specimens with the size of 50 x 150 mm were prepared and sewn along all edges of the test fabric.

The test was carried out using Atlas Launder-Ometer Model LEF Serial No. LO-3843. The test specimens were washed at the temperature of 49 ± 2 ^{o}C for 45 min or over a specific period of time with 0.15 % the 1993 AATCC Standard Reference Detergent WOB (without optical brightener) of total volume of 150 ml and with 50 steel balls.

Specimens were then rinsed three times with de-ionized water and removed excess water before drying at the temperature lower than 71 ^{o}C in an air circulating oven. All specimens were conditioned at standard humidity and temperature before further evaluating.

Chapter 4: The Exploration of Natural Dyes and Their Coloration on Textiles Mainly Focused on Curcumin Natural Yellow Dye

4.1 Introduction

Curcumin is a main active constituent of a compound extracted from turmeric root. Its structure is 1,7-bis(4-hydroxy-3-methoxyphenyl)- 1,6-heptadiene-3,5-dione, containing two symmetrical sequences of conjugated double bond systems of feruloylmethane as shown in the Figure 4.1 below (Goel, *et al.*, 2008).

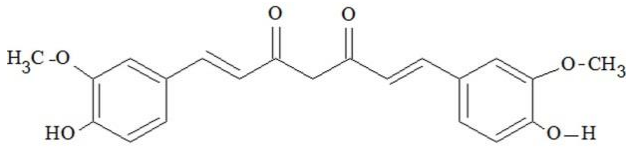


Figure 4. 1 Chemical structure of curcumin in keto form.

Turmeric (*Curcuma longa Linn*) is a tropical perennial herb belonging to the Zingiberaceae (ginger) family. It can grow naturally or being cultivated in India and several countries in South and Southeast Asia including certain regions in Africa, America, and Pacific Ocean Islands. The trend toward its overall productivity has been increasing especially in India (Ravindran, 2007). Therefore, it is likely to be an abundant natural resource for curcumin natural dye for textile applications.

As mentioned in previous chapter, there are two major chemical constituents obtained from turmeric extraction; volatiles and non-volatiles. Volatile oils are normally secondary metabolites of aromatic plant with very complex natural mixtures (Ravindran, 2007). They are sometimes called essential oil and can be extracted using several methods. They are, as examples, hydro-distillation (Silva, *et al.*, 2005), steam distillation (Manzan, *et al.*, 2003), solvent extraction (Manzan, *et al.*, 2003; Nishiyama, *et al.*, 2005), and newly introduced

supercritical fluid extraction (Chang, *et al.*, 2006).

Non-volatiles are curcuminoids consisting of curcumin as a major component and its 3 derivatives: demethoxy curcumin, bis-demethoxy curcumin, and latest identified cyclocurcumin (Ravindran, 2007). Curcuminoids are well known as a curcumin colorant (Jayaprakasha, *et al.*, 2005). They are typically extracted after the isolation of volatile oils.

In this chapter, all experiments had been conducted to manifest the full exploitation of turmeric, a natural resource of the dye and all approaches selected were environmental friendly and not destructive. Firstly, volatile oils were isolated from dry turmeric powder by steam distillation method. Then curcumin dye was extracted using ethanol at room temperature, followed by dyeing applications of curcumin on both natural and synthetic fibers using traditional methods. The volatile oils and curcumin dye were characterized for their chemical components and compared with references. Dyed samples were measured for their color strength and color characteristics. Color fastness to washing was also evaluated.

4.2 Experimental

4.2.1 Turmeric Oil Extraction

Approximately 500 grams of turmeric powder was put into a fabric bag and placed on the perforated plate in the biomass flask. The steam distillation apparatus was assembled as shown in Figure 4.2 and a 5 L heating mantle was used as a heating source. To generate a quick steam, water was heated at the maximum rate until it started to boil. Then, the heating was lowered to a medium

level and remained at this level for 3 hours. After boiling for a certain time, the steam and distillate were condensed and collected at the receiver. Since the layer of volatile oil and condensed water were separated, the volatile oil layer was conveniently collected. Then, it was analyzed for its composition using the Hewlett-Packard GC 6890 series coupled to the Agilent Technologies 5973 MSD system.

Figure 4. 2 Steam distillation apparatus.

4.2.2 Curcumin Extraction

The turmeric residue from steam distillation was extracted using a ratio of 1:10 of a turmeric residue to N-hexane at room temperature for 3 hours to get rid of

the remaining volatile oil and resin. The liquid portion was then decanted to separate the organic solvent from the solid particles and the remaining of N-hexane in the particles was evaporated. The dry solid particles were placed in absolute ethanol to extract curcumin at room temperature for overnight. The analysis of isolated product was carried out with FT-IR.

4.2.3 Curcumin Dyeing application

4.2.3.1 Pretreatment of Textile Substrates

All textile substrates were scoured with 0.5 g/l non-ionic surfactant (Diawin®EWN) at 60 ^{o}C for 30 minutes. After scouring, they were rinsed thoroughly and then dried at 35 ^{o}C.

4.2.3.2 Curcumin Dyeing

The curcumin stock solution of 0.1 % (w/v) was prepared using ethanol-water solvent mixture (2:3 v/v for cotton, acrylic and PLA fibers and 1:9 v/v for polyester fiber). 2.0 grams of each substrate were dyed at 6 different shades (based on 6 dye concentrations: 0.25, 0.5, 0.75, 1.25, 1.0, and 1.5 % o.w.f.) using a fiber to dye liquor ratio of 1:15. The pH of dye bath was adjusted to 5.0±0.2 with acetic acid solution. The dyeing temperature was set for each fiber as follows: 90 ^{o}C for cotton, 100 ^{o}C for acrylic and PLA fibers, and 130 ^{o}C for polyester fiber, and the temperature was kept constant for 60 minutes.

After the completion of dyeing step, the bath was cooled down to 60 ^{o}C and the dyed sample was taken out and well rinsed with warm water, followed with cold water. Dyed sample was afterwards washed in a solution containing 0.5 g/l of

non-ionic surfactant (Diawin®EWN) at a fiber to liquor ratio of 1:25 for acrylic fiber and 1:15 for the others at 60 ^{o}C for 30 minutes.

4.2.3.3 Color Measurement

After dyed substrates were stored in a conditioning room for 24 hours, their spectral reflectance data were measured at the wavelength between 400-700 nm with the Scope System (from Gain Associate Inc.) using Macbeth CE-7000A spectrophotometer under illuminant D65, 10^{o} standard observer with UV component included and specular component excluded for polyester and acrylic fibers and included for cotton and PLA fibers. A graph relationship between *K/S* value at maximum absorption wavelength for individual fiber and curcumin concentrations was established. *L*a*b** values of all dyed samples were also reported. In the CIE 1976 *L*a*b** color space, the vertical *L** axis represents lightness of the object color, ranging from 0-100 where *L** =0 for darkest color and *L** = 100 for lightest or brightest color. The horizontal axes represent *a** and *b**values, perpendicular to each other, where *a**>0 for red, *a**<0 for green, *b**>0 for yellow, and *b**<0 for blue.

4.3 Results and discussion

4.3.1 Turmeric Oil Extraction

The distillate coming out from the extraction was a milky pale yellow liquid as shown in Figure 4.3. It was analyzed for determining organic components using the Hewlett-Packard GC 6890 series coupled to the Agilent Technologies 5973 MSD system. 0.005 gram of the condensate from the experiment was diluted in 1 ml of absolute ethanol. 5 µL of the solution was injected with the splitless

technique into the gas chromatographic column. The compound was identified by matching with those of NIST 08.L (60 libraries) and Wiley 275.L (40 libraries) and published mass spectra. Its spectrum is shown in the Figure 4.4 below.

Figure 4. 3 Distillate obtained from the steam distillation extraction of turmeric powder.

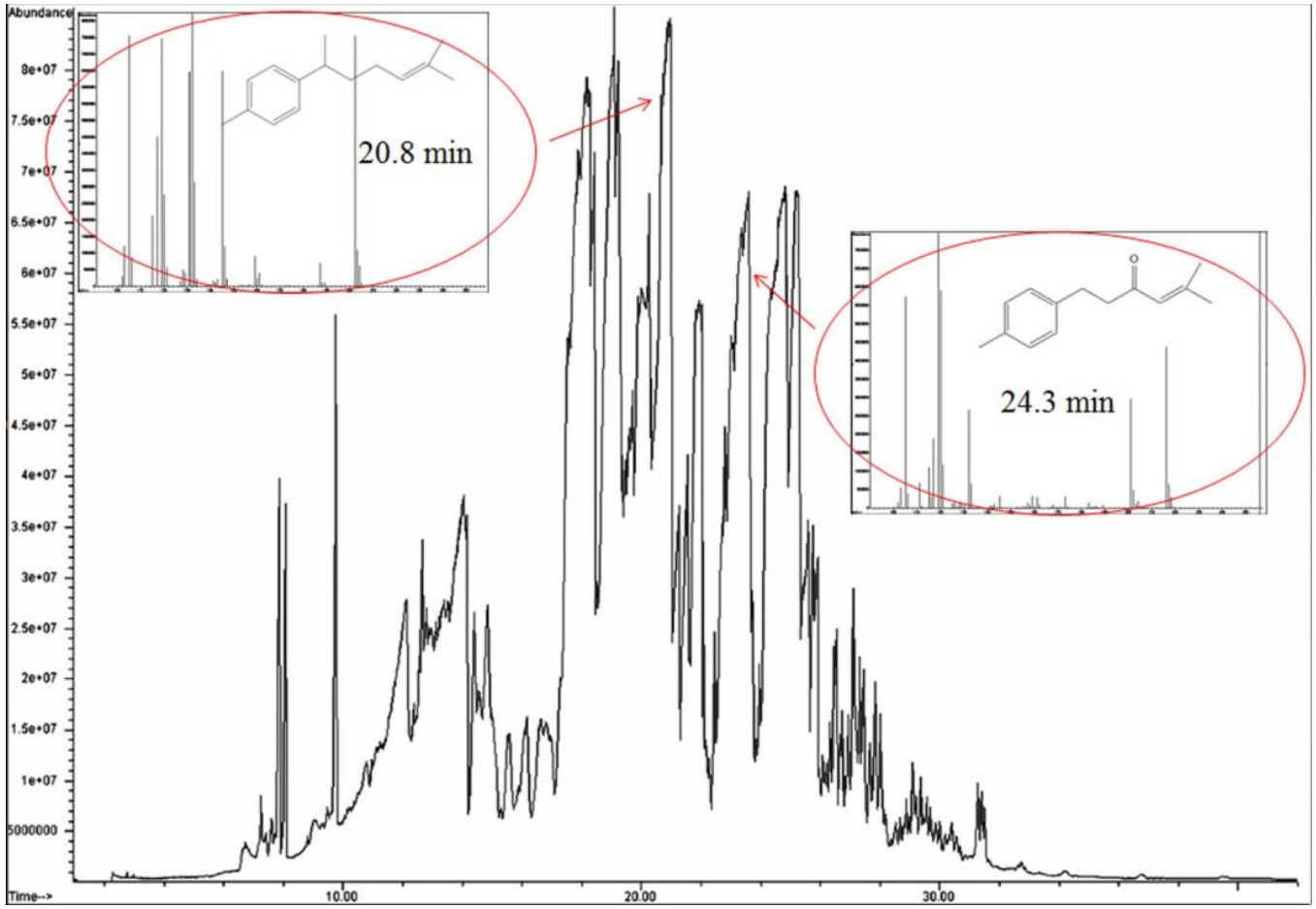


Figure 4. 4 GC-MS Chromatogram, TIC, of Volatile Oil Distilled from Turmeric Powder from Thailand. The peaks at 20.8 and 24.3 minutes show Curcumene and Ar-turmerone, as identified by the mass spectrum.

The GC-MS analysis of the distillate from dry turmeric powder exhibited only 2 prominent and sharp peaks. They were detected at 20.8 minute and 24.3 minute retention times and being identified as curcumene or Benzene, 1-(1, 5-dimethyl-4-hexenyl)-4-methyl- and ar-turmerone, respectively. The mass spectrum of each compound is shown in Figure 4.4.

The analysis result showed that curcumene and ar-turmerone are two major compounds detected by Hewlett-Packard GC 6890 series coupled to the Agilent Technologies 5973 MSD system. Both curcumene and turmerone are classified as a sesquiterpenoids. Ar-turmerone has been always identified as a major component in turmeric oil extracted by different techniques. Chatterjee, *et al.* (2000) used organic solvent extraction method to isolate volatile oil from turmeric in India. The amount of turmerone and ar-turmerone was 70 % of all compositions identified in the essential oil. Asghari, *et al*. (2009) also found that ar-turmerone accounts for the highest amount for all compositions extracted from *Curcuma longa L*. from Thailand by hydrodistillation method. Moreover, Gopalan, *et al.* (2000) used supercritical carbon dioxide to extract oil from turmeric (*Curcuma longa L*.). The most abundant component identified from the steam distilled oil was ar-turmerone. Thus, it can be concluded that the extract obtained from the experiment is the volatile oil.

4.3.2 Curcumin Extraction

After steam distillation, the residue was placed in N-hexane for removal of the volatile oil and resin, then in absolute ethanol to obtain curcumin. The product had reddish brown in color and it was slightly sticky due to the remaining resin. Therefore, the product was cast into films for FT-IR analysis and the result was

compared with that of the commercial curcumin. Its spectra were scanned on the wave number between 4000 and 450 cm^{-1}. The result is shown in Figure 4.5

FTIR Results of Extracted Curcumin

The extracted curcumin was dissolved in dichloromethane and the solution was dropped onto a dry KBr circular plate. After dichloromethane was evaporated, a thin film of extracted curcumin was formed on the plate and it was analyzed for an infrared absorption spectrum using FTIR analysis.

FTIR Results of Commercial Curcumin

Powder of commercial Curcumin was mixed and milled homogenously with potassium bromide and then compressed into a thin pellet using KBr press for FTIR analysis. Its spectra were scanned on the wave number between 4000 and 450 cm^{-1}. The result is shown in Figure 4.5

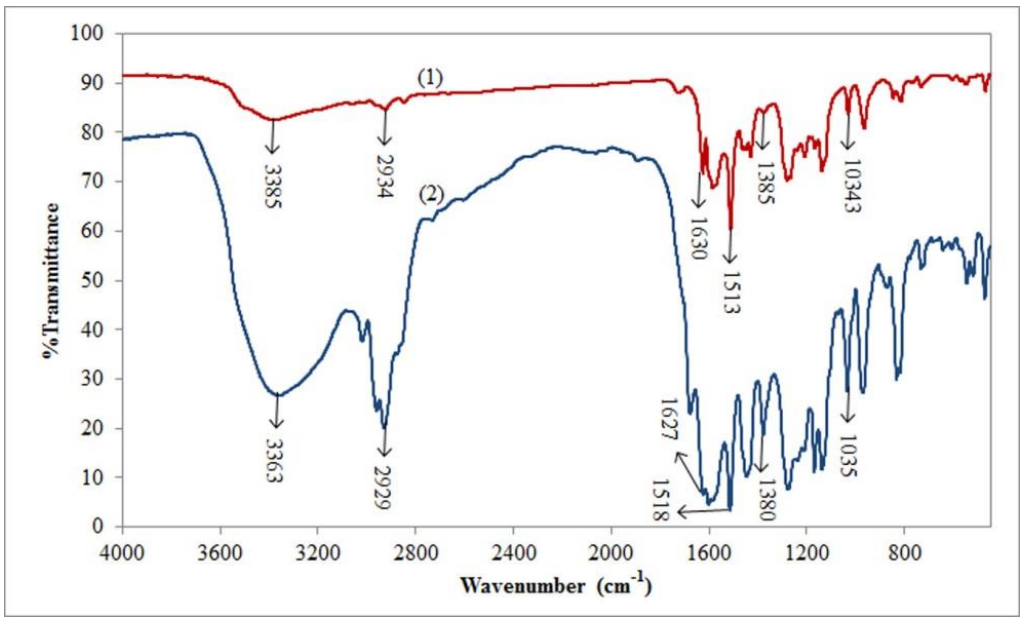


Figure 4.5 FT-IR spectra of commercial curcumin (1) and extracted curcumin (2).

From Figure 4.5, FTIR spectra were assigned and described as follows. The FTIR spectrum of commercial curcumin showed the highest frequency at 3378 cm^{-1} indicated the stretching of both free hydroxyl groups ν (O-H). The band with medium and weak intensities in the region at 1430 cm^{-1} and between 782-729 cm^{-1} was assigned to the in-plane and out-of-plane deformations of hydroxyl (OH) group, respectively. The stretching of C-O was also observed at 1239 $cm^{-1}$and the C-O-H bending was prominent at the region of 1511 cm^{-1}. Weak and variable intensities between the region 2924 cm^{-1} were attributed to the C-H stretches of aromatic ring of the curcumin. Due to aromatic rings and alkene structures of the curcumin, the following bands were observed and assigned. The most prominent band at 1585 cm^{-1} was from the stretch of C=C of the benzene ring and alkene groups, whereas the band at 1376 cm^{-1} was due to the bending of two methyl(CH_3) groups. The C-H out-of-plane bending was observed at 886, 856, and 815 cm^{-1} indicating meta substituted aromatic rings. The medium intensity of the C-H in-plane deformation vibrations of phenyl ring (CCH) was at 1185 cm^{-1}.

For two methyl groups, the C-H stretche at the region of 2924 cm^{-1} was accountable for. The CH_3 and CH_2 bending at 1376 and 1450 cm^{-1}, respectively, were also distinctive to indicate the functional group. Apart from the stretching of C-O at 1239 cm^{-1}, two bands with medium intensity at 1031 cm^{-1} and sharp with strong intensity at 1282 cm^{-1} were assigned as C-O-C symmetrical and asymmetrical stretching, respectively. According to the experiment, commercial curcumin was analyzed in solid form. A strong band at 1625 cm^{-1} was observed and attributed to C=O vibrations of the diketo form.

After the identification of commercial curcumin was confirmed, the comparison

with curcumin extracted from the experiment was made. The result is presented in Table 4.1.

Table 4. 1 Wavenumbers of main vibration modes from infrared spectral data of commercial curcumin and extracted curcumin. Vibrational modes: (ν) stretching; (δ) in-plane bending; (—) not observed.

Assignment	ν_{IR} (cm^{-1})	
	Commercial Curcumin (Solid Method)	Extracted Curcumin (Cast Film)
ν(O-H)	3378	3363
ν(C-H) sp3	2924	2929
ν (C=O)	1625	1624
ν(C=C)	1585	1602
ν(C=C)	1511	1514
δ (C-O-H)	1511	1514
δ(-CH_2)	1450	1447
δ (O-H)in-plane	1430	-
δ(-CH_3)	1376	1378
ν (C-O)	1239	1237
ν_{as}(C-O-C)	1282	1278
δ (C-H) in-plane	1185	1169
ν_s(C-O-C)	1031	1034
δ (C-H) out-of-plane	886, 856, 815	832,819
δ (O-H)out-of-plane	782-729	876,779

From Table 4.1, it was concluded that the extracted product was curcumin. Generally, comparison results should be better when both results are obtained from using the same procedure. However, the result from the solid method for curcumin as a standard substance is more apparent when comparing the absorption frequencies of certain functional groups of the extracted product.

Thus, the methodology of how to acquire curcumin colorant from turmeric rhizome can be concluded here that the process should consist of two consecutive steps, i.e., the turmeric oil extraction has to be initially carried out and followed by curcumin extraction process.

4.3.3 Curcumin Dyeing Application

4.3.3.1 Dyeing Results

Four fibers (cotton, polyester (PET), poly(lactic acid), and acrylic) were each dyed in a concentration series of curcumin solution in solvent mixture of ethanol and deionized water. Indicative colors of the dyed fibers are shown in Table 4.2. Color parameters of dyed fibers, *K/S* and CIE *L*a*b**, were measured using the GretagMacbeth CE 7000A spectrophotometer. CIE *L* a* b** coordinates of 4 fibers dyed at various concentrations of curcumin dye are presented in Table 4.3 and a plot of color strengths at the maximum absorption wavelength of individual fibers against a given series of curcumin dye concentrations was made and shown in Figure 4.6. Moreover, a plot of a*b* values was also plotted to compare the difference of color coordinates among the four dyed fibers and presented in Figure 4.7.

Table 4.2 Indicative colors of 4 fibers dyed at various concentrations of curcumin dye.

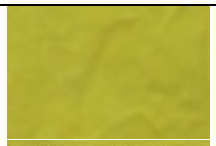
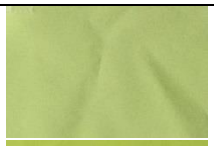
Concentration (% o.w.f.)	Type of fibers			
	Acrylic	PLA	Polyester	Cotton
0.5 %				
0.75 %				
1.25 %				
1.5 %				

Table 4.3 CIE *L* a* b** coordinates of 4 fibers dyed at various concentrations of curcumin dye.

Concentration (% o.w.f.)	*L**				*a**				*b**			
	Acrylic	PLA	Polyester	Cotton	Acrylic	PLA	Polyester	Cotton	Acrylic	PLA	Polyester	Cotton
0.50	85.9	82.1	90.0	84.0	-18.9	0.0	-16.2	7.0	69.5	77.3	63.0	73.7
0.75	84.8	82.0	88.3	82.5	-17.9	0.4	-14.4	9.1	82.3	77.6	75.2	76.2
1.25	83.6	82.0	84.8	81.9	-17.2	0.7	-9.6	10.1	83.7	77.8	84.9	77.6
1.50	83.8	81.7	84.8	80.3	-16.2	1.3	-8.4	12.2	87.0	76.1	85.8	78.7

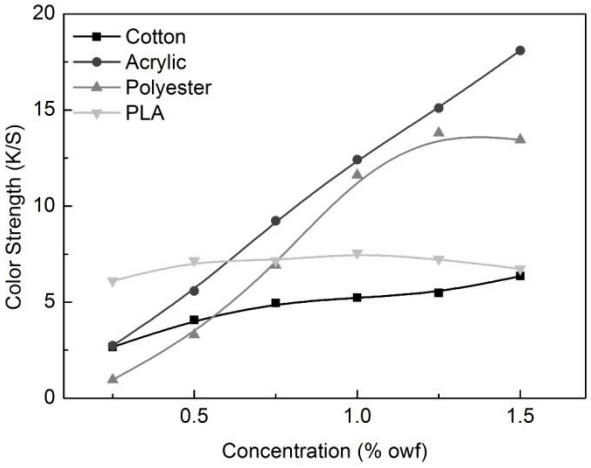


Figure 4. 6 Color strength at the maximum absorption wavelength of individual fibers dyed at various concentrations of curcumin dye.

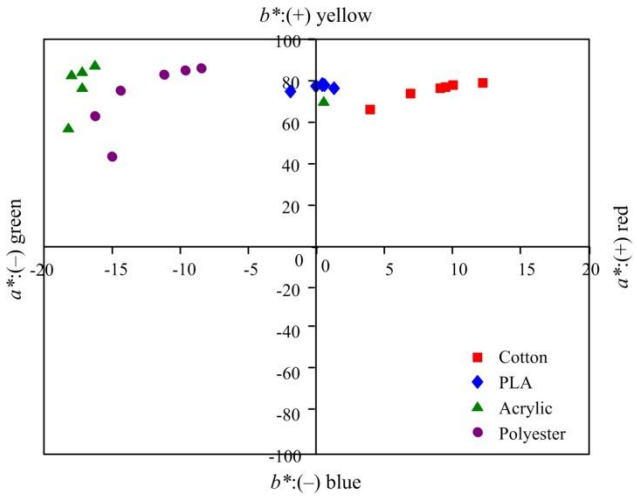


Figure 4. 7 *a*b** coordinate values of 4 fibers dyed at various concentrations of curcumin dye.

From the photos shown in Table 4.2, all of the dyed fabrics and yarns were of the same yellow color but with different hues. According to the color measurement, the maximum absorption wavelengths for all dyed fibers were slightly different,

430 nm for acrylic and polyester fibers, 440 nm for poly(lactic acid) fiber and 450 nm for cotton fiber.

As shown in Figure 4.6, the color strength (*K/S*) values of dyed fibers at their maximum absorption wavelength were increased with increasing of curcumin concentration, but the increasing rate was different for each dyed fiber. It was quite high for acrylic and polyester dyeing, but considerably smaller for cotton and poly(lactic acid) dyeing. Generally, initial dye concentration of a dye solution affects the adsorption capacity greatly and higher dye concentration has greater driving force to accelerate the diffusion of the dye from the solution into fiber (Chairat, *et al.*, 2005; Chiou & Li, 2002; Liu, *et al.*, 2010; Özer & Dursun, 2007). The adsorption of curcumin dye on fibers possibly depends on physical adsorption rather than chemical adsorption like the adsorption of tannic acid on cotton and acrylic fibers. The adsorption may be due to the formation of hydrogen bonding between polar group of curcumin and polar group present along the polymer chain of each fiber such as polar nitrile group of acrylic fiber and hydroxyl group of cotton fiber (Shokry, *et al.*, 2013). When no further adsorption occurs, the saturation point is reached and it is different for individual fibers depending on their internal accessible volume (Ibbett, *et al.*, 2006; Mahmoud, *et al.*, 2012). In this dyeing experiment, saturation point for each dyeing was obviously observed except for acrylic fibers.

In Table 4.3, *L*a*b** values of all dyed samples were collectively presented. *L**values of all samples were similarly bright regardless of fiber type and tended to decrease with increasing curcumin dye concentrations. Dyed cotton and PLA showed positive *a** and *b**, while dyed polyester and acrylic showed positive *a**

and negative *b**. From a plot of *a***b** color coordinates presented in Figure 4.7, *a**, *b** values of all fibers were shifted along *a** axis towards red coordinate and slightly down along *b** axis toward blue coordinate when concentration of curcumin increased. These data were corresponded to color visual assessment as illustrated in Table 4.2. Color shade change was noticeable. Dyed samples turned to be redder and bluer as curcumin concentration increased.

4.3.3.2 Color fastness to Laundering

Table 4. 4 Color fastness to laundering of various fibers dyed with curcumin according to AATCC 61:2010.

Fiber types	Gray Scale for Color Changes	Gray Scale for Color Stain
	under Illuminant D65/Observer 10^{o}	
Cotton	1	2-3
PLA	1-2	2-3
Polyester	4	4-5
Acrylic	4	4-5

Color fastness to laundering results of dyed fibers presented in Table 4.4 clearly showed that curcumin dyed on cotton and PLA fibers displayed very poor fastness, whereas both dyed polyester and acrylic fibers were good fastness to laundering.

In general, curcumin has substantivity toward cotton fibers. The molecular structure of curcumin is well-conjugated and co-planar; therefore it can penetrate into the cotton fiber and aggregate on the surface of cellulosic chains. During fiber dyeing, more curcumin molecules should diffuse into the fiber since water solubility of curcumin increases with increasing the dyeing temperature. However, the attraction forces between curcumin and cotton should be Van der Waals forces and intermolecular hydrogen bonds between phenolic groups of curcumin and hydroxyl group of cotton (Ammayappan and Moses, 2009). Both Van der Waals and the intermolecular forces between molecules are generally much weaker than bonds (Heaton, 1994; Reger, *et al.*, 2009). Therefore, curcumin dyes on cotton can be easily washed off, leading to poor color fastness

to washing.

According to the previous dyeing results, curcumin could also provide good dyeing results on all synthetic fibers. This is due to the fact that curcumin has the structure and certain properties similar to disperse dyes. Disperse dyes are applied not only to polyester fibers, their applications to PLA and acrylic fibers have been reported as well. Accordingly, it is possible to achieve good dyeing results on synthetic fiber dyeing with curcumin. The mechanism of dyeing curcumin on these synthetic fibers may be explained by means of the solution mechanism as follows.

Curcumin has extremely low water solubility at room temperature. At higher temperature, the breakage of intramolecular hydrogen bonds within the curcumin increases and, therefore, its water solubility increases (Jagannathan, *et al.*, 2012; Rukmani & Sundrarajan, 2012). Therefore, a small amount of curcumin may become an aqueous solution at high temperature in dyebath, whereas the greater proportion of curcumin is still dispersed. When curcumin particles dissolve in water, individual curcumin molecules should be obtained and they should be small enough to be adsorbed onto the surface of the fiber. After adsorption, these molecules diffuse from the surface into the interior of the fiber. Before reaching the dyeing equilibrium which is constant under a dyebath condition, curcumin particles from the bulk dispersion dissolve in the depleted aqueous curcumin solution to replenish individual molecules of curcumin. These molecules can be further adsorbed on to the fiber surface (Burkinshaw, 1995).

During dyeing at high temperature, synthetic polymer chains can undergo segmental motions and the degree of these motions increase with increasing the

dyeing temperature. At the temperature above *Tg*, free volume of the fibers is more accessible. As a result, curcumin molecules in aqueous solution should be able to diffuse readily into the fiber until a saturation point is reached. Then curcumin may be adsorbed and agglomerated to physically form an ordered monolayer or multilayer film mainly at the surface of the polyester chains. Since the dyeing mechanism of curcumin on synthetic fibers including PET and PLA is similar to that of disperse dyes, linear Nernst isotherm should be the most appropriate sorption model corresponding to its dyeing behavior (Wu, *et al.*, 2013).

In comparison between polyester and PLA, their dyeing behaviors were different from each other, although their chemical structures are comparatively similar. This is because PLA is an aliphatic polyester while PET is an aromatic polyester. Both fibers show different refractive index. PLA has lower refractive index than PET, and therefore the deeper color of dyed PLA should be the result of its lower refractive index property (Lunt & Bone, 2001). In terms of color fastness to laundering, fibers with higher hydrophilicity in nature could possibly show lower fastness to laundering. As PLA is relatively more hydrophilic than PET (Burkinshaw, 2016), more water molecules may access to curcumin dye molecules located inside the fiber, leading to a dye migration from the fiber. In addition, curcumin molecules also have less attraction for PLA. With all these phenomena, dyed PLA showed lower color fastness to laundering than dyed PET.

4.4 Conclusions

In this work, an application of dry turmeric powder for fiber dyeing was used to fully demonstrate natural resource utilization. Turmeric powder was steam

distillated to remove volatile oil, cited in literature as a useful compound for certain applications. The extract from steam distillation was further treated in ethanol to obtain curcumin colorant. As curcumin has limited in water solubility at neutral pH and at room temperature, ethanol was used to facilitate the dyeing application. Four fibers containing cotton, PET, PLA, and acrylic were dyed under specific conditions and dyed fibers were analyzed for various properties. Visual assessment and the color values obtained from spectrophotometer measurement were presented and expressed their correlation. Referring to dyeing results, curcumin was affirmed for its application in textile coloration as a natural colorant for synthetic fibers with unexpectedly good washing fastness except for PLA. However, its affinity to cellulosic fibers has to be developed.

Chapter 5: Synthesis of Modified Curcumin with N-protected Glycine

5.1 Introduction

Curcumin is a substantive natural dye. It has been long used for textile coloration previously only on natural fibers. It also has been known for various biological properties such as antibacterial activity. After applied to cotton and wool, curcumin can still exhibit its antibacterial activity (Han & Yang, 2005; Reddy, *et al.*, 2013). However, a few disadvantages have been noted especially for dyeing on cotton fibers. That is, its affinity and fastness properties are relatively poor. Another is from its intrinsic property. It has limited water solubility causing lower biological activity and difficulty in use. Accordingly, a number of studies have been attempted using different techniques and technologies to solve the problems, mainly focused on extraction and dyeing processes. For examples, the application of gamma and UV radiation was carried out to improve the dyeability of curcumin on cotton fibers (Adeel, *et al.*, 2011; Bhatti, *et al.*, 2010). Air atmospheric plasma treatment and UV excimer lamp irradiation were employed to investigate the improvement in dyeing application of curcumin on polyester fabric (Kerkeni, *et al.*, 2012). The effect of different mordants and mordanting methods was also extensively studied in terms of color strength, color shade, fastness properties, and antibacterial activities. The use of mordants could improve the dyeing qualities of curcumin on cotton and provide a variety of colors (Sachan & Kapoor, 2007; Umbreen, e*t al.*, 2008). Nevertheless, most mordants are metallic salts. Long-run impact on the environment has to be concerned. High accumulation level of elements from metallic mordants such as alum can cause harm to living species. For example, high concentration of aluminium is toxic to aquatic freshwater organisms (Ward, *et al.*, 2006).

Therefore, the chemical modification of curcumin is of great interest, since the chemically modified curcumin exhibited better properties and functions while used in food and medicinal areas (Parvathy, *et al.*, 2010).

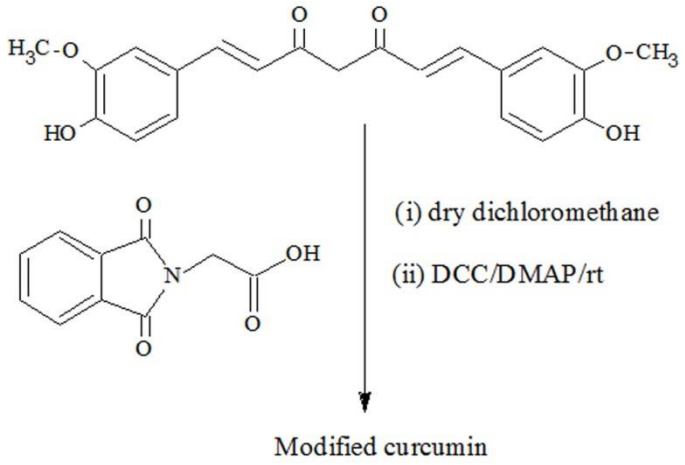
For the chemical modification of curcumin, it is well noted that the important functional group playing an important role in reacting with other reactants is phenolic group. This functional group can participate in a number of chemical reactions including nucleophilic substitution with carboxylic acids, acyl chlorides, and acid anhydrides under basic conditions (Rawn, 2014). Amino acids are organic compounds containing the carboxyl group as a major component. Therefore, the reaction of curcumin with an amino acid was of great potential to fulfill the purpose of this experiment. Carboxyl group of an amino acid may undergo the esterification reaction with a phenolic group of curcumin when amino group is protected. After the completion of the reaction, the amine protecting group of the amino acid has to be deprotected. Then free amino group of the modified product would be available and water solubility of the product could be enhanced.

In this chapter, N-protected glycine was selected to react with curcumin in the presence of a base catalyst and a hydrating agent. This reaction produced dicyclohexylurea as a by-product which was difficult to remove completely. Accordingly, N-protected glycine was substituted by a higher reactive N-protected glycine chloride to react with curcumin. The product was preliminarily characterized for its molecular structure prior to applying to polyester fabric to study its properties.

5.2 Experimental

5.2.1 Synthesis of Modified Curcumin with N-phthaloylglycine

N-phthaloylglycine (102.58 mg; 0.5 mmol) and commercial curcumin (184.2 mg; 0.5 mmol) were put in a 100 ml 3 neck round bottom flask. 10 ml of dry dichloromethane was added to dissolve both reactants and the mixture was stirred for a few minutes. A few drops of DMF were added to make the mixture completely soluble. Dicyclohexylcarbodiimide (DCC) (196 mg; 0.95 mmol) and 4-Dimethylaminopyridine (4-DMAP) (61 mg; 0.5 mmol) were dissolved in 15 ml dry dichloromethane before adding gradually into the reactant solution. The mixture solution was stirred at room temperature for overnight. The scheme of the synthesis reaction is shown below.



Scheme 5. 1 Synthesis route of the reaction between curcumin and N-phthaloylglycine.

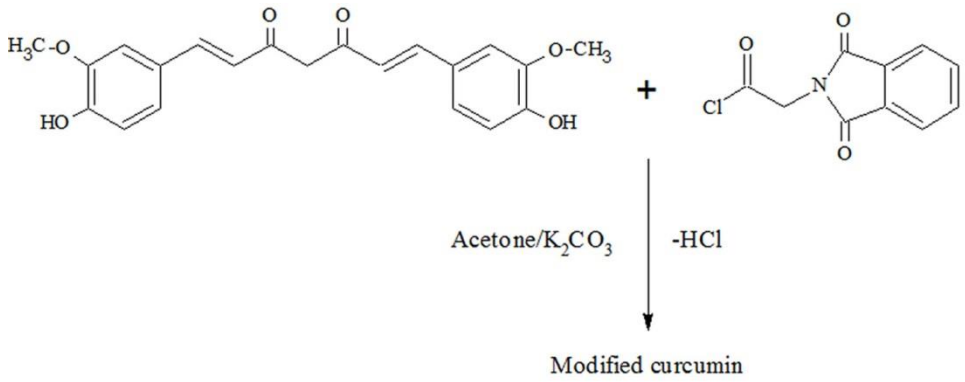
After the completion of the reaction was monitored by TLC on silica gel with 1,2-dichloroethane/chloroform/ethanol:4.0/0.2/0.1, the reaction solution was filtered to remove the precipitate using a filter paper. The filtrate was dried to

nearly completion. The remaining residue was then diluted with ethyl acetate and subsequently added to a separatory funnel. A certain amount of deionized water was added into the funnel to partition the remaining dicyclohexylurea by-product. Then the organic layer was collected and evaporated. The product in the organic layer was purified by TLC on silica gel using 1,2-dichloromethane/ chloroform/ethanol(4.0/2/0.2, v/v) as a solvent system. The chemical structure of the purified product was investigated with an electrospray ionization mass spectrometry operated with positive ion detection mode; *m/z;* 556 ($M\text{-}H^+$).

5.2.2 Synthesis of Modified Curcumin with N-phthalylglycyl Chloride

Curcumin (73.6 mg; 0.2 mmol) and K_2CO_3 anhydrous (55.28 mg; 0.4 mmol) were put in a 100 ml 2 neck round bottom flask equipped with a magnetic stirrer. 10 ml of dry acetone was added to dissolve both reactants and the mixture was stirred for a few minutes. N_2 gas was purged for 5 minutes. N-phthalylgylcyl chloride (89.4 mg; 0.4 mmol) was dissolved in 5 ml dry 1,2-dichloroethane before adding dropwise into curcumin solution at 0 ^oC in an ice bath and then the mixture solution was stirred at room temperature for overnight. The completion of the reaction was monitored on TLC using 1,2-dichloroethane/chloroform/ ethanol: 4.3/0.6/0.1 as the eluent system. Then reaction mixture was filtered to remove solid particles. The filtrate was dried before re-dissolving in chloroform. The aqueous solution of 0.1 % (w/v) NaOH was subsequently added into the filtrate solution to extract the remaining curcumin using the separatory funnel. The product was further purified using TLC with 1,2-dichloroethane as an eluent. The major component was taken and characterized by ^1H NMR and ESI-MS. ^1H NMR ($CDCl_3$): δ (ppm) = 3.95 (s, 6H, OCH_3), 4.32 (s, 2H, $N\text{-}CH_2$), 7.77-7.92

(m, 4H, Ar-H); m/z; 556 ($M-H^+$).



Scheme 5. 2 Synthesis route of the reaction between curcumin and N-phthalylglycylchloride.

5.3 Results and Discussion

5.3.1 Characterization of Modified Curcumin from the Reaction of Curcumin with N-phthaloylglycine

5.3.1.1 Mass Spectroscopy

After the synthesis reaction was complete, the product was purified and then analyzed by ESI-MS using positive ion mode. The spectrum is presented in Figure 5.1.

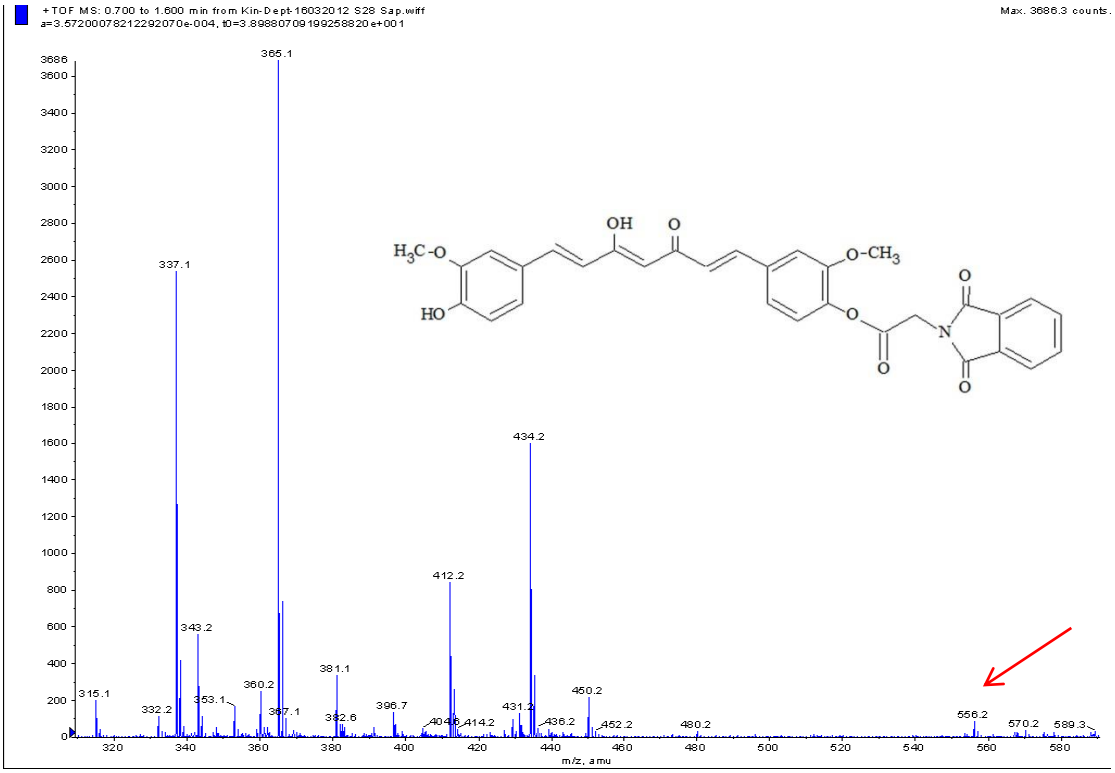


Figure 5. 1 ESI-MS spectrum of the synthesis product from the reaction of curcumin and N-phthaloylglycine.

From the mass spectrum of the product in Figure 5.1, only one peak at *m/z* 556 could be assigned as a compound of interest. It may be 1,7-Bis (4-o-glycinoyl-3-methoxy phenyl)-1,6-heptadiene-3,5-dione. The possible structure is proposed and presented in Figure 5.2 as follows.

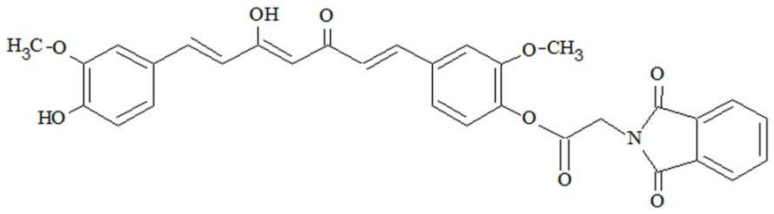
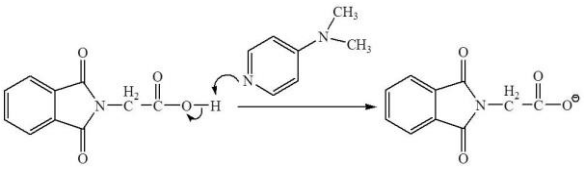


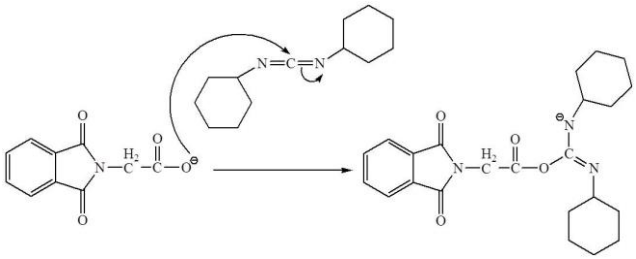
Figure 5. 2 Structure of modified curcumin from the reaction of curcumin and N-phthaloyl glycine, the product of interest.

In this synthesis, N, N'-dicyclohexylcarbodiimide (DCC) and 4- dimethylamino pyridine (DMAP) were used as a hydrating/coupling agent and nucleophilic base catalyst, respectively. The reaction of curcumin and N-protected glycine using DCC/DMAP was possibly the Steglich esterification. The general mechanism of the catalyzed reaction to produce modified curcumin is proposed and depicted in Figure 5.3.

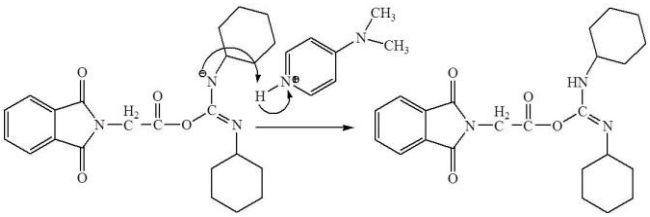
Step I



Step II



Step III



Step IV

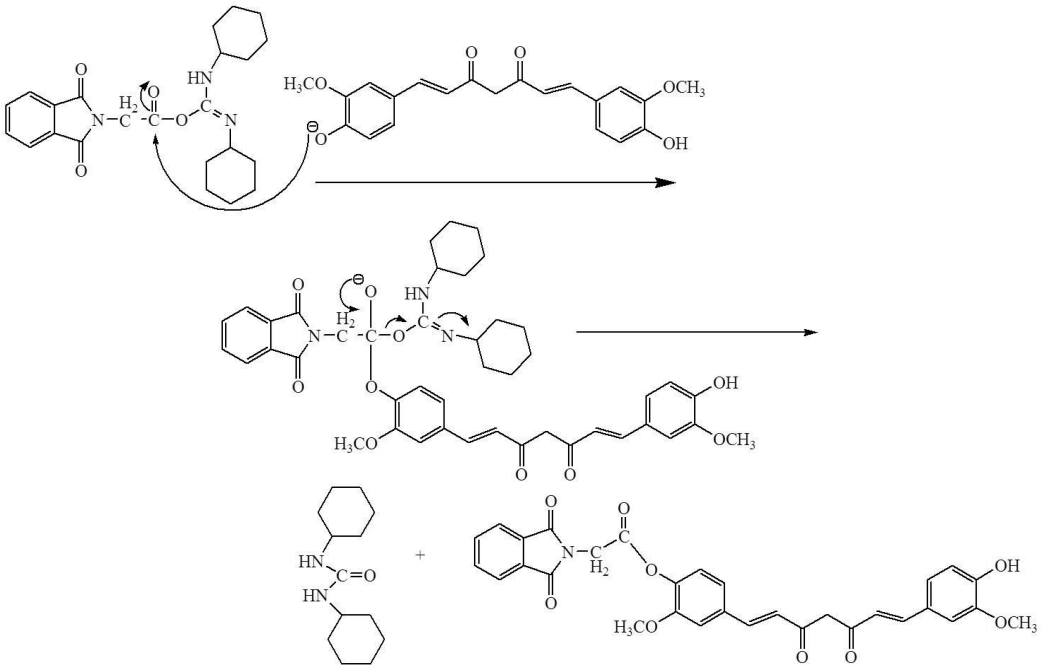


Figure 5. 3 Proposed mechanism of the reaction of curcumin and N-phthaloylglycine in the presence of DCC/DMAP.

As presented in Figure 5.3, DMAP is highly nucleophilic at nitrogen atom of the ring. It could deprotonate the carboxyl group of N-protected glycine as seen in step I. The deprotonated N-protected glycine subsequently reacted with DCC to form an O-acylisourea intermediate as presented in step II. Then DMAP was deprotonated by the intermediate O-acylisourea (step III). The intermediate was more reactive to react with ionized phenolic group of curcumin to produce ester products with dicyclohexylurea (DCU) by-product (step IV) (Alsehli, 2011; Iwasawa, *et al.*, 2007).

The purified product was also investigated further by NMR. However, the removal of dicyclohexylurea (DCU) by-product was practically not simple. The purification of the target compound, therefore, was not sufficient to achieve a satisfied NMR spectrum. Accordingly, no further experiment was conducted, although the condition of this reaction was considered mild and could be conducted at room temperature due to DCC/DMAP. To pursue the study, N-protected glycine was substituted by a more reactive N-protected glycine chloride.

5.3.2 Characterization of Modified Curcumin from the Reaction of Curcumin with N-phthalylglycyl Chloride

5.3.2.1 Mass Spectroscopy

The product from the reaction of N-protected glycine chloride and curcumin was purified before analyzing for its molecular mass by TOF mass spectroscopy. The spectrum of the compound is presented in Figure 5.4.

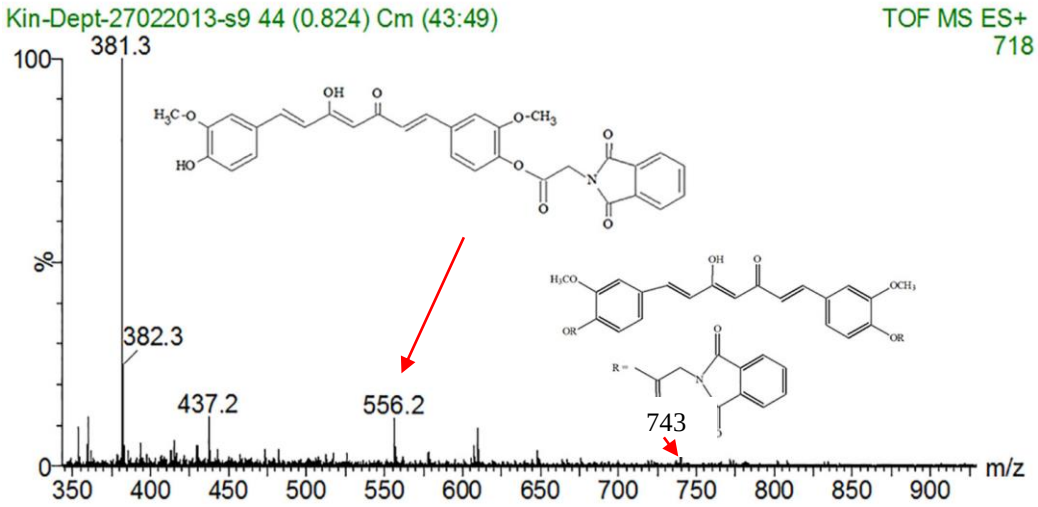
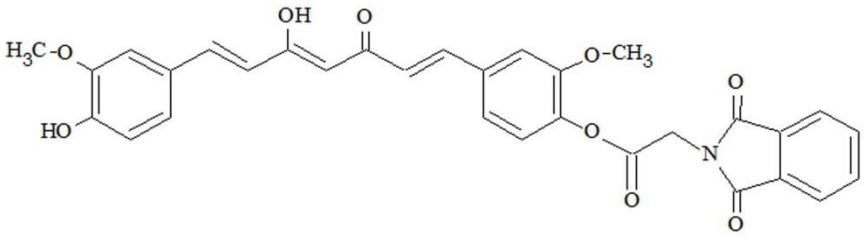
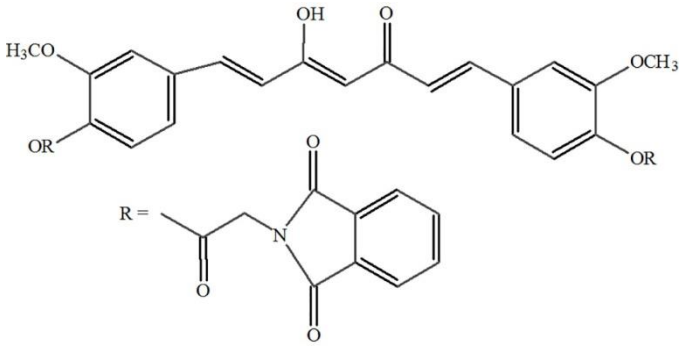


Figure 5. 4 TOF MS mass spectrum and the structural formula of modified curcumin.

As shown in Figure 5.4, TOF mass spectrum of the major compound showed molecular ion peak at m/z 556, corresponding to monoester of curcumin with the molecular weight of 555.53. The structure of the compound of interest is proposed and depicted in Figure 5.5 (a).



(a)



(b)

Figure 5. 5 Structures of the products of interest from the reaction of curcumin and N-phthalylglycyl chloride. (a) monoester curcumin; (b) diester curcumin.

However, another component was also found in this reaction as revealed by its mass to charge ratio at 743. This component could be identified as a diester of curcumin and its structure is proposed and written in Figure 5.5 (b). Compared to the major component found at m/z 556, its abundance was considerably less.

5.3.2.2 NMR

Curcumin and the purified product were diluted in chloroform solvent (CDC_{l3}) and then structurally characterized with NMR technique. The spectra of both compounds were presented in Figure 5.6.

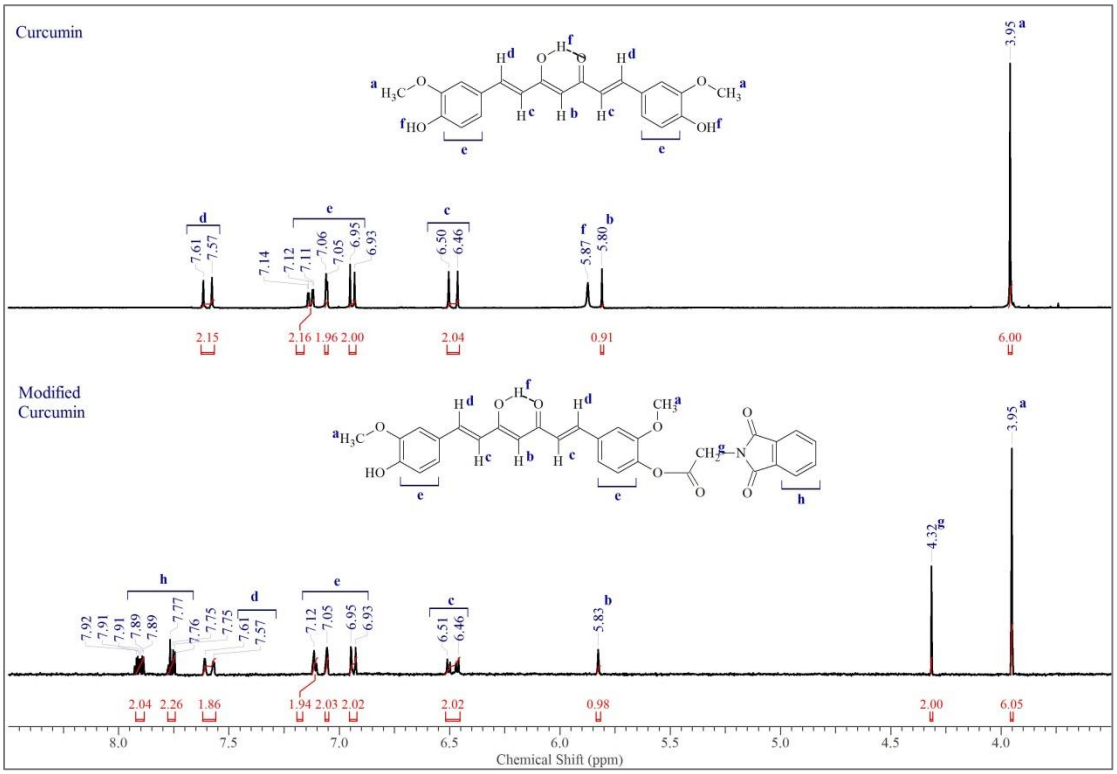


Figure 5. 6 ^{1}H NMR spectra of curcumin and its derivative in chloroform.

From the ^{1}H NMR spectrum of the modified structure, new peaks or chemical shifts were detected as follows. A singlet for two protons of (CH_2 linked to nitrogen) was found at 4.32 ppm. Typical multiplet from aromatic ring of N-phthaloyl protected group was observed for four protons at 7.77-7.92 ppm. The structure of original curcumin still retained as observed in Figure 5.5. A singlet at 3.95 ppm was observed for six protons of two methoxy groups. A singlet at 5.83 ppm was observed for proton of enol or keto group, multiplet at 6.93-7.12 ppm for benzene ring, 6.46-6.51 ppm for protons of C_2 and C_6, and 7.57-7.61 for protons of C_1 and C_7 of conjugated system.

The interpretation of ^{1}H NMR spectrum of the modified curcumin was in consistence with the ^{1}H NMR spectrum of curcumin and the result of mass spectrum analysis previously discussed. The mechanism of the reaction of

curcumin and N-protected glycine chloride is proposed as follows.

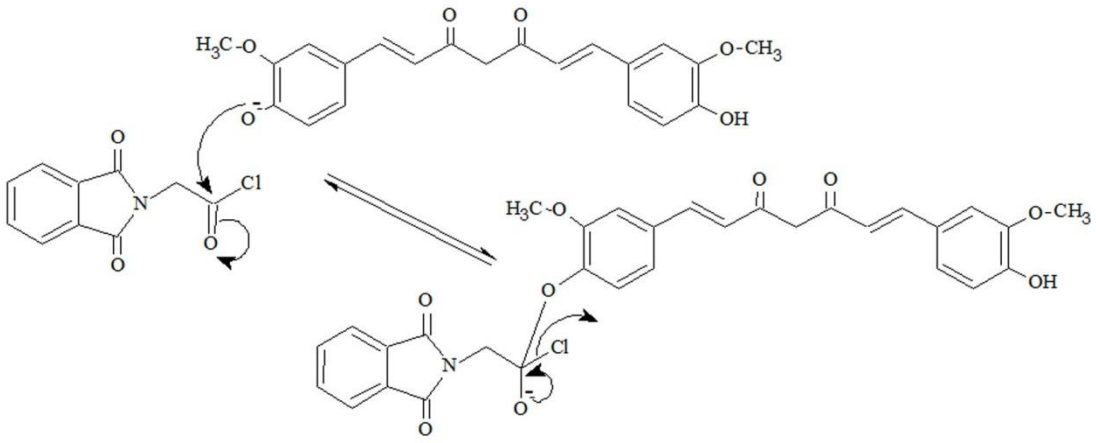


Figure 5. 7 Proposed mechanism for the reaction of modified curcumin.

The mechanism is likely to be nucleophilic addition/elimination reaction since molecule of N-phthalylglycyl chloride is very active toward nucleophilic attack. With the presence of anhydrous potassium carbonate in acetone, phenolic group of curcumin would form a corresponding phenolate anion, an effective nucleophile for the reaction and it could attack at positively charged C-atom of the amino acid chloride. An ester compound could be produced with an elimination of chloride atom as proposed in Figure 5.7.

5.3.2.3 UV-visible Absorption Spectroscopy

The product with N-phthaloyl protective group was further investigated for other properties such as absorbance properties, instead of the modified curcumin with free amino group due to difficulty in removal of the protecting group by ammonia/pyridine. UV-visible spectroscopy was employed to investigate the absorbance characteristics of the product, compared to that of the curcumin reactant and the results are shown in Figure 5.8 below.

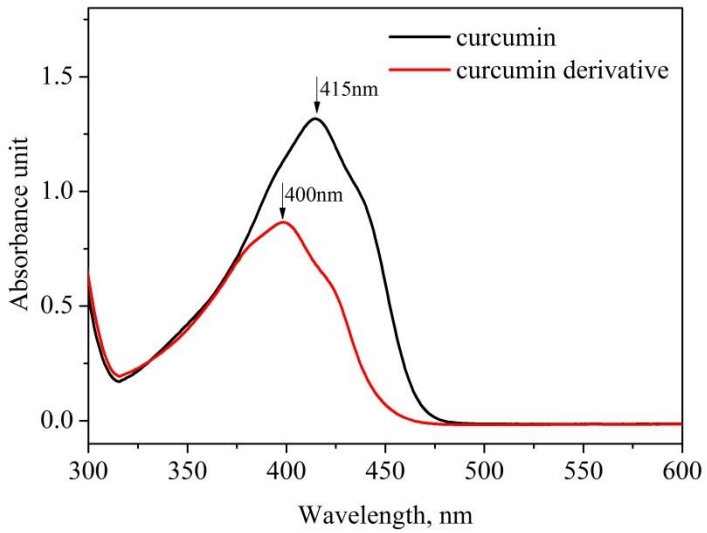


Figure 5.8 UV-visible absorption spectra of curcumin and its derivative in chloroform.

As seen in Figure 5.8, curcumin in chloroform exhibited the maximum absorption at 415 nm with the molar extinction coefficient value (ε_{molar}) of 1.3149 $M^{-1}cm^{-1}$,whereas the maximum absorption wavelength of the modified curcumin was at 400 nm with ε_{molar} of 0.8944 $M^{-1}cm^{-1}$. Compared to curcumin, the maximum absorption wavelength and intensity of the modified curcumin were decreased indicating the phenomenon of hypsochromic shift (blue shift) and hypochromic effect.

The structure of the modified curcumin elucidated by the analysis of MS and NMR was possibly a monoester in which the ester bond connected to one phenolic moiety of curcumin. In principle, lone pair electrons at oxygen atom of phenolic groups of curcumin can extend the electron delocalization by sharing an electron to the conjugation system of benzene ring (Mander & Liu, 2010). When proton of phenolic group was substituted and became an ester moiety, electron

resonance was more prominent at the ester bond; that is, lone pair electrons on the oxygen atom (-O-C=O) from previous phenolic group would be delocalized over the carbon and oxygen atoms (-O-C=O) of the ester bond rather than distributing to the adjacent benzene ring. The distribution of lone pair electrons on the oxygen atom to pi-conjugated system of the benzene ring of the modified curcumin may not exist. As a result, the energy difference between HOMO and LUMO would be larger. More energy would be needed for their atoms or molecules to be excited to higher energy level and shorter wavelengths of the light would be absorbed, causing hypsochromic shift (blue shift) as seen in Figure 5.8.

5.3.3 Dyeing Application

Based on the result of the structure analysis, the entire product was applied to polyester fabric. Its structure exhibited relatively more hydrophobicity compared to the original structure of curcumin and it resembled disperse dyes. Accordingly, both curcumin and modified curcumin were dyed on polyester fabric under the same dyeing conditions. The dyed fabrics were measured for their color strength and other color coordinates using Macbeth CE-7000A spectrophotometer. The color strength values at a given series of concentrations of each dye were then plotted as a function of visible wavelength and the results are presented in Figures 5.9 and 5.10. The CIE L^* a^* b^* coordinates of both dyes were shown in Table 5.1.

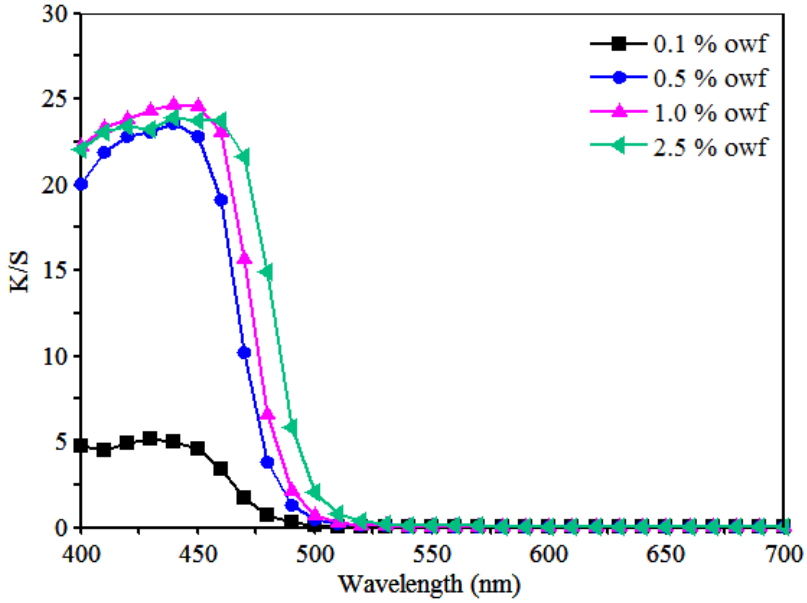


Figure 5. 9 Plot of the color strength values at a given series of curcumin concentrations as a function of visible wavelengths.

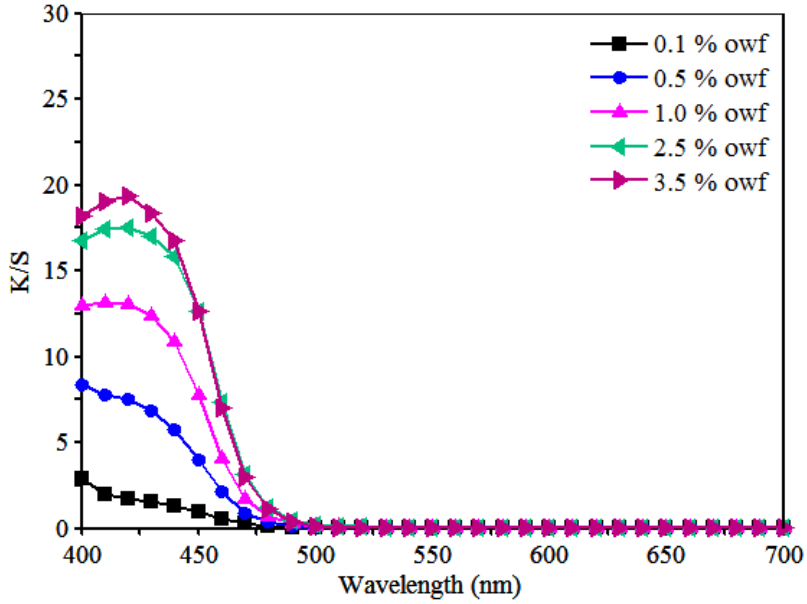


Figure 5. 10 Plot of the color strength values at a given series of modified curcumin concentrations as a function of visible wavelengths.

The color strength presented in both Figures 5.9 and 5.10 was calculated from fractional reflectance of the dyed sample at a particular wavelength using the

Kubelka-Munk equation as follows:

$$K/S = (1 - R_\infty)^2 / 2R_\infty$$

where K and S are the absorption and scattering coefficients, respectively, of a substrate or dye and R_∞ is the reflectance of a color sample of infinite thickness.

From Figures 5.9 and 5.10, graph of *K/S* against wavelength of curcumin was different from that of modified curcumin in that graph of curcumin was not identical in shape for all concentrations. There was an existence of crossing between the graph of 1.0 and 2.5 % o.w.f. indicating shade change occurrence as concentration increased. Graph lines were nearly overlapped at the concentration of 0.5 % o.w.f. and higher, implying that its saturation concentration was reached. This unusual deviation of the graph of *K/S* against visible wavelength of curcumin was likely to indicate its critical characteristic. It could create problems when curcumin was used in combination.

Table 5. 1 CIE *L* a* b** coordinates of curcumin and modified curcumin at a given series of concentrations.

Concentration (% o.w.f.)	*L**		*a**		*b**	
	Modified Curcumin	Curcumin	Modified Curcumin	Curcumin	Modified Curcumin	Curcumin
0.1	87.9	84.1	-15.5	-16.1	38.8	65.0
0.5	86.3	81.1	-16.7	-5.4	62.6	96.6
1.0	84.6	81.6	-14.7	-9.5	72.5	92.1
2.5	83.0	77.4	-11.6	5.3	79.7	97.1

From CIE *L** *a** *b** coordinates given in Table 5.1, modified curcumin had higher value of parameter *L** (lightness), more negative *a** value (greenness) but less positive *b** value (yellowness) when compared to curcumin. It could be indicated that the color shade of curcumin and modified curcumin was slightly different. Modified curcumin was lighter in appearance with much greener and bluer color in effect.

5.3.4 Antibacterial Activity Evaluation

After dyed with curcumin and modified curcumin, polyester fabrics were evaluated for antibacterial activity against *S. aureus* according to ASTM E2149 and the results are reported in Table 5.2.

Table 5. 2 Assessment of antibacterial activity on polyester fabric dyed with curcumin and modified curcumin against *S. aureus*: ASTM E2149

Fabric Samples	Visual Assessment	Reduction in CFU (%)
Undyed control fabric		36.00
Curcumin-dyed fabric (2.5 % o.w.f.)		94.61
Modified Curcumin-dyed fabric (3.5 % o.w.f.)		92.30

As seen in Table 5.2, polyester substrate as a blank or control fabric showed 36.0 % reduction in bacteria growth, whereas % reduction of the curcumin-dyed fabric and modified curcumin-dyed fabric was 94.61 and 92.30, respectively.

The decrease in number of colony forming units of polyester control fabric is possibly due to titanium dioxide typically added into polyester fibers for delustering purposes. As commonly known, rutile titanium dioxide also has photocatalytic activity capable of producing radicals that effectively prevent the growth development of microbes. Hence, titanium dioxide presented in polyester fibers may influence the growth of bacteria.

As known, curcumin belongs to phenolic compound group. It has two phenolic groups in a molecule. Phenolic compounds possess antibacterial properties by causing leakage of bacteria cell constituents leading to its death afterwards. Therefore, curcumin-dyed fabric exhibited the ability in reducing the growth of bacteria due to the presence of phenolic group in curcumin molecules. For modified curcumin, only one phenolic group was remained as indicated by MS and NMR characterization. As a result, the ability to reduce the growth of bacteria of the modified curcumin-dyed fabric even at the highest concentration of dye (3.5 % o.w.f.) was lower than that of the curcumin-dyed fabric at lower concentration (2.5 % o.w.f.).

5.3.5 Color fastness to Laundering

Table 5. 3 Color fastness to laundering of curcumin-dyed polyester and modified curcumin-dyed polyester according to AATCC 61-2010.

Fabric	Gray Scale Rating for Color Change	Gray Scale Rating for Color Staining					
		Acetate	Cotton	Nylon	Silk	Viscose rayon	Wool
Curcumin dyed	4-5	5	5	3-4	5	5	5
Modified Curcumin dyed	5	4-5	5	4	5	5	5

The color fastness to laundering of polyester fabric dyed with curcumin and modified curcumin was conducted according to AATCC 61: 2010 using the standard laundering conditions no.2A. The results were acceptable as illustrated in Table 5.3. The wash fastness ratings for both color change and staining on individual component bands of multifiber test fabric were mainly 4-5 (between slight change/stain and no change/stain) or 5 (no change/stain) except for color staining on nylon.

5.4 Conclusions

In this experiment, the modification of curcumin was attempted to gain more advantages for textile application. In the first effort, N-phthaloyl glycine was selected to react with curcumin in the presence of N,N'-dicyclohexylcarbodi imide (DCC) as a dehydrating agent and dimethylaminopyridine (DMAP) as a catalyst. The reaction tended to undergo nucleophilic substitution reaction at room temperature. The monoester curcumin was obtained with the formation of dicyclohexylurea DCU by product. However, the difficult removal of DCU was encountered. Then the substitution of N-protected glycine acid chloride was considered as a more reactive reactant. N-phthaloylglycyl chloride was selected to react with curcumin under N_2 atmosphere. A better result was achieved. The structure of the new modified compound could be characterized and identified. Since the structure of the new compound was more hydrophobic compared to the original structure, the dyeing application on polyester fabric was studied. The evaluation of color fastness to washing and antibacterial activity of dyed fabric was carried out. The color of dyed fabric was bright yellow. The color fastness to laundering was good to all fibers of multifiber test fabric. The antibacterial activity of the modified curcumin-dyed fabric was slightly lower than that of curcumin dyed fabric.

However, removal of protecting group from the synthesized product was more difficult than what mentioned in references. The use of ammonia and pyridine could hydrolyze the ester bond linked between phenolic group of curcumin and carboxyl group of protected amino acid. Accordingly, no further investigation was carried out for this modification reaction.

Chapter 6: Synthesis of Modified Curcumin with Glycidyl-trimethylammonium Chloride

6.1 Introduction

Curcumin, a beneficial component from turmeric, is rather limited in textile applications especially on cotton due to poor water solubility and lack of its affinity toward the fiber. A great number of studies were indeed performed to widen and strengthen the applications of curcumin in textile coloration. In previous experiment, the synthesis of a curcumin monoester was carried out by nucleophilic addition/elimination reaction using N-protected glycine and N-protected glycine chloride. Certain difficulties were encountered due to the ease of hydrolysis of the amino acid chloride. Therefore, another reagent was selected to replace the use of the amino acid chloride. It was a quaternary ammonium cationizing reagent (QAC). QACs have been widely used in the reaction with hydroxyl or amino-bearing molecules such as polyvinyl alcohol, hemicellulose, starch, chitin, and chitosan to impart cationic moieties into their backbone structures. Examples of quaternary ammonium cationizing reagents are glycidyltrimethylammonium chloride (GTMAC) and 3-chloro-2-hydroxy propyltrimethylammonium chloride (CTAC). This group of reagents is comparatively more practical since it is commercially available with reasonable price. Another advantage of using this reagent is related to the reaction conditions. This reaction is usually conducted in an aqueous media with the presence of alkaline as a catalyst. The condition is considered mild and environmental friendly. Principally, cationic product can be simply separated from the reaction solution by precipitating out with the addition of a short chain alcohol like ethanol.

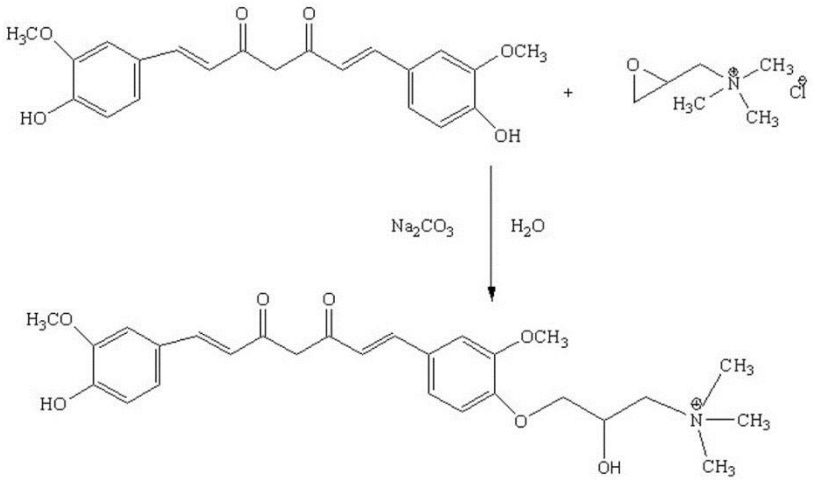
In this study, glycidyltrimethylammonium chloride (GTMAC) commonly referred to as an epoxide was selected to chemically react with the phenolic group of curcumin. GTMAC could introduce positive charges to the molecules of curcumin as seen in Scheme 6.1 and different properties of curcumin with the cationic moiety were potentially expected to be observed after being applied to textile substrates.

6.2 Experimental

6.2.1 Synthesis of Modified Curcumin with Glycidyltrimethylammonium Chloride

Curcumin (0.92 g; 2.5 mmol) and anhydrous sodium carbonate (0.26 g; 2.5 mmol) were loaded in a 100 ml 2-neck round bottom flask containing a magnetic stirring bar. 15 ml of deionized water was added to dissolve sodium carbonate and curcumin was subsequently dissolved in the alkaline solution. The curcumin solution was then heated up to the temperature of 60 ^{o}C before gradually adding the aqueous solution of glycidyltrimethylammonium chloride (0.76 g; 5.0 mmol in 10 ml of DI water) into it. The reaction temperature was held constantly at 60 ^{o}C for 24 hours prior to cooling down the reaction mixture to room temperature. Then, 0.5 % (v/v) of formic acid was added to neutralize the mixture. A large enough volume of absolute ethanol was added to the mixture to precipitate the product, which was isolated afterwards by centrifugation. The precipitant was then washed several times with the absolute ethanol to remove other substances especially curcumin reactant. The product was dried at room temperature. The overall reaction yield (%) was 42.73 %. The modified product was analyzed by mass spectroscopy. Its chemical structure was suggested based on the molecular

ion m/z [M-Cl + H] at 520.



Scheme 6. 1 Synthesis route of modified curcumin from curcumin and glycidyltrimethylammonkum chloride.

6.2.2 In Vitro Cytotoxicity Test

Cytotoxicity is the degree to which an agent possesses a specific destructive action on certain cells (Dorland, 2011). It is related to cellular injury or death caused by soluble mediators, immune cells, and drugs or toxicants (Hodgson, *et al.*, 1988). Different methods for the assessment of cytotoxicity are available (Barile, 2013). One of the most commonly used methods is MTT assay. MTT or 3-[4, 5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide is a water soluble tetrazolium salt. It can be converted to purple formazan crystals by succinate dehydrogenase in mitochondria of viable cells. In general, viable cells with active metabolism have constant mitochondria activity. Accordingly, mitochondria activity can be reflected by the conversion of MTT into a purple colored formazan crystal.

When cells die, they lost the ability to convert MTT into formazan. Therefore, the quantity of formazan crystals is directly proportional to the number of living cells. However, the formazan product deposited as a precipitate on both inside and outside of the cells is insoluble in water. To solubilize the product, a second reagent such as DMSO is needed to generate a uniform purple color solution. Then the absorbance of the solution can be recorded at the wavelength of 570 nm by a plate reading spectrophotometer. The color formation can serve as a useful and convenient marker of only the viable cells.

The MTT assay is widely used in cell viability and cytotoxicity tests since it is easy to use, safe and highly reproducible. It was the first homogenous cell viability assay developed for a 96-well plate. It can measure viable cells in plates in relative high-throughput without the need for elaborate cell counting (Riss, *et al.*, 2013).

Cell Culture

The normal human skin fibroblasts cells (ATCC: CRL 2522) were selected to be cultured cells for the cytotoxicity test of GTMAC-modified curcumin. Cells were cultivated in Dulbecco's Modified Eagles medium (DMEM) supplemented with 10 % fetal bovine serum and maintained at 37 ^{o}C under a 95 % humidified atmosphere and 5 % CO_2 for 3-5 days or until the cells reached (entered into) logarithmic phase of cell growth.

The cells were mechanically detached and suspended in the medium before inoculating them into 96-well tissue culture plates. In each well, 100 µl of the cell solution containing 1.0 x 10^4 cells was added. Then the plates were incubated at 37 ^{o}C with 5 % CO_2 for 24 hours.

In Vitro Cytotoxic Activity

An initial stock concentration of 2,000 ppm of GTMAC-modified curcumin was prepared by dissolving in 1:1 solvent mixture of ethanol and deionized water. Then a given series of the modified curcumin concentrations were made from the initial concentration using a serial dilution. Each dilution was added to the cells and the cells were then incubated for 24 and 48 hours before evaluating for cytotoxic effects. Six replicate wells were used for each test concentration. The same procedure was performed for the solvent mixture as the control experiments.

At the end of incubation, the cell media was removed prior to adding 25 µl of MTT solution (5 mg/ml) to the cells including control ones and incubated for 4 hours until the purple precipitate became visible. Then dimethyl sulfoxide (DMSO) was added into each well to solubilize the formazan crystals which were formed in the mitochondria. The absorbance of each formazan solution was then read at a wavelength of 570 nm to determine cell viability. The results were expressed as mean values ± standard deviation of six measurements. The relative cell viability (%) was calculated by the following equation:

Cell viability (%) = (Absorbance of sample/Absorbance of control) x 100

6.2.3 Skin Irritation Test

Three young adult albino rabbits with healthy intact skin were chosen to be subjected to the skin irritation test according to ISO 10993-10:2010(E). Fur of individual rabbits on both sides of their spine (approximately 10 x 15 cm) was shaved 24 hours before the test. Their bare skin was used as the application sites for the test and the locations are illustrated in Figure 6.1.

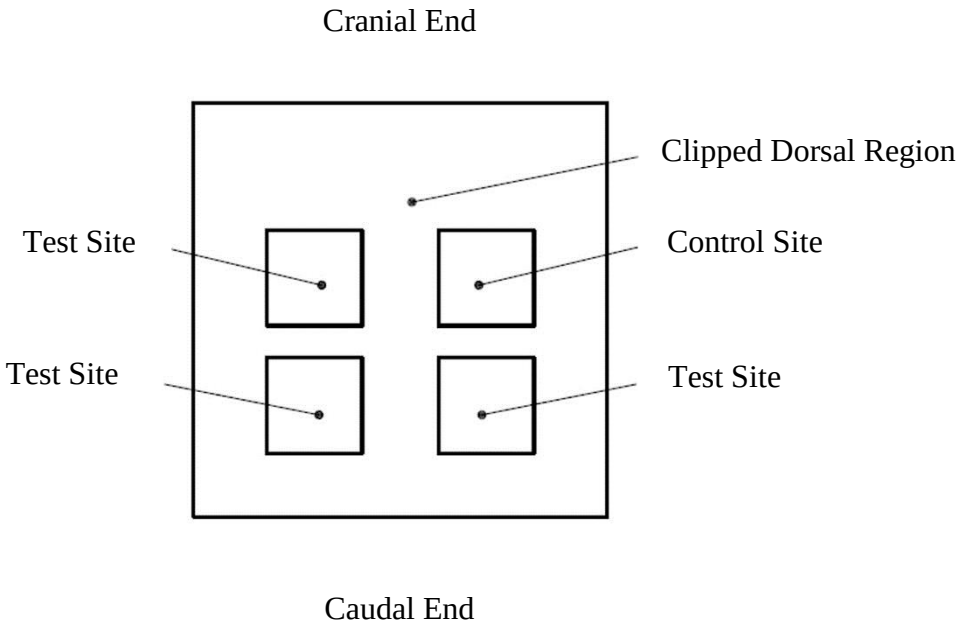


Figure 6. 1 Location of skin application sites on a rabbit (ISO 10993-10:2010(E)).

The test sample was the cotton fabric dyed with GTMAC-modified curcumin. The average of *K/S* value of the dyed fabric was 1.919 at 440 nm. Two pieces of the test sample with a dimension of 2.5 x 2.5 cm were prepared and moistened with 0.9 % sodium chloride solution before patching on test sites on the skin of each rabbit. Two pieces of gauze patch dampened with the same saline solution were also patched on the rabbit's bare skin at control sites. Individual sites were then wrapped with a bandage for a minimum contact time of 4 hours.

After the termination of exposure, all test samples and blank controls were removed. All application sites were washed with lukewarm water. Then each site was observed and noted for its appearance at 1, 24, 48 and 72 hours after the patch removal. The irritation score for erythema and eschar formation and oedema formation was given using the scoring system for skin reaction as shown in Table 6.1.

Table 6. 1 Scoring system for skin reaction (ISO 10993-10:2010(E)).

Reaction	Score
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate erythema	3
Severe erythema (beet-redness) to eschar formation preventing grading of erythema	4
Oedema formation	
No oedema	0
Very slight oedema (barely perceptible)	1
Well-defined oedema (edges of the area well-defined by definite	2
Moderate oedema (raising approximately 1 mm)	3
Severe oedema (raised more than 1 mm and extending beyond the area of exposure)	4
Total possible score for primary irritation	8

For individual rabbits, all erythema grades and oedema grades at 24, 48, and 72 hour exposure from both observation sites were totaled separately for each test sample and control. The summation of the irritation score for individual rabbits was then divided by 6 (two test observation sites, three time points). The average score of each test sample was afterwards subtracted by the average score of control test. This calculated value of each rabbit was added together before being divided by the number of rabbits as shown in the equation below.

PII

$$= \left[\frac{\sum_{i=1}^{3}(Er+Ed)_{i,24h}+(Er+Ed)_{i,48h}+(Er+Ed)_{i,72h}}{6}\right]T - \left[\frac{\sum_{i=1}^{3}(Er+Ed)_{i,24h}+(Er+Ed)_{i,48h}+(Er+Ed)_{i,72h}}{6}\right]C/3$$

Where,

T, treated; C, control; Er, erythema; Ed, oedema; i, the number of rabbits

Description of the primary or cumulative irritation index is given in Table 6.2.

Table 6. 2 Primary or cumulative irritation index categories in a rabbit (ISO 10993-10:2010(E)).

Response category	PII
Negligible	0-0.4
Slight irritation	0.5-1.9
Moderate irritation	2-4.9
Severe irritation	5-8

6.3 Results and Discussion

6.3.1 Characterization of GTMAC-modified Curcumin

6.3.1.1 Mass Spectroscopy

After the product of the reaction between curcumin and glycidyltrimethyl ammonium chloride was obtained, ethanol was used to purify and separate the modified product. The product was then analyzed by Quadrupole Time-of-Flight mass spectrometer configured with an electrospray ionization source. The mass spectrum of the product is shown in Figure 6.2.

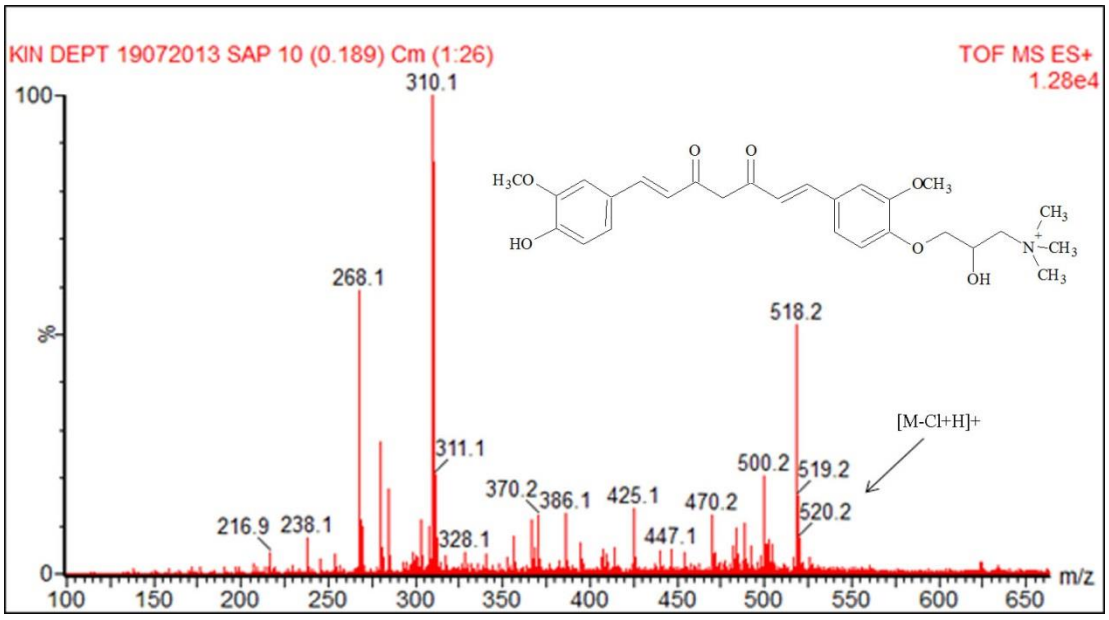


Figure 6. 2 Mass spectrum of GTMAC-modified curcumin.

By analyzing mass of the product, the peak corresponding to the GTMAC-modified curcumin was at m/z ratio of 520, with positive mode of ionization. This m/z value corresponded to the structure as depicted in Figure 6.3 and name of the GTMAC-modified curcumin was abbreviated as CurGTMAC.

In this reaction, sodium carbonate aqueous solution was used to activate phenolic groups of curcumin into corresponding phenolate ion or nucleophilic alkoxide. Under alkaline condition, this nucleophile would attack unsymmetrical epoxide ring of GTMAC at the least substituted carbon and opened the epoxide to form the modified curcumin as illustrated in Figure 6.3.

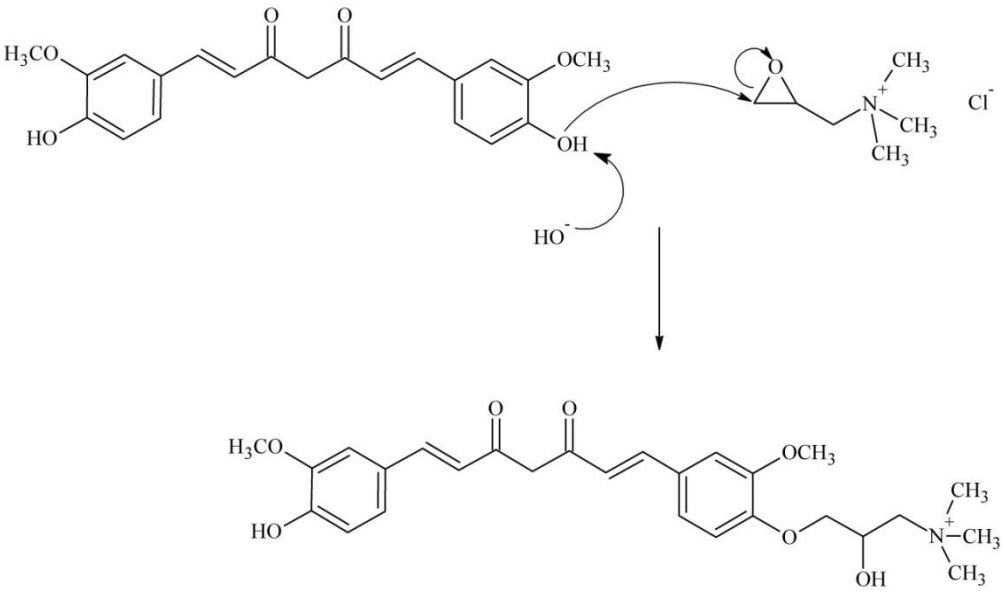


Figure 6. 3 Proposed mechanism of the reaction between curcumin and GTMAC in alkaline aqueous solution.

6.3.1.2 FT-IR

Powder of CurGTMAC was mixed and milled homogenously with potassium bromide and then compressed into a thin pellet using KBr press for FTIR analysis. Its spectra were scanned on the wave number between 4000 and 450 cm^{-1}. The result is shown in Figure 6.4

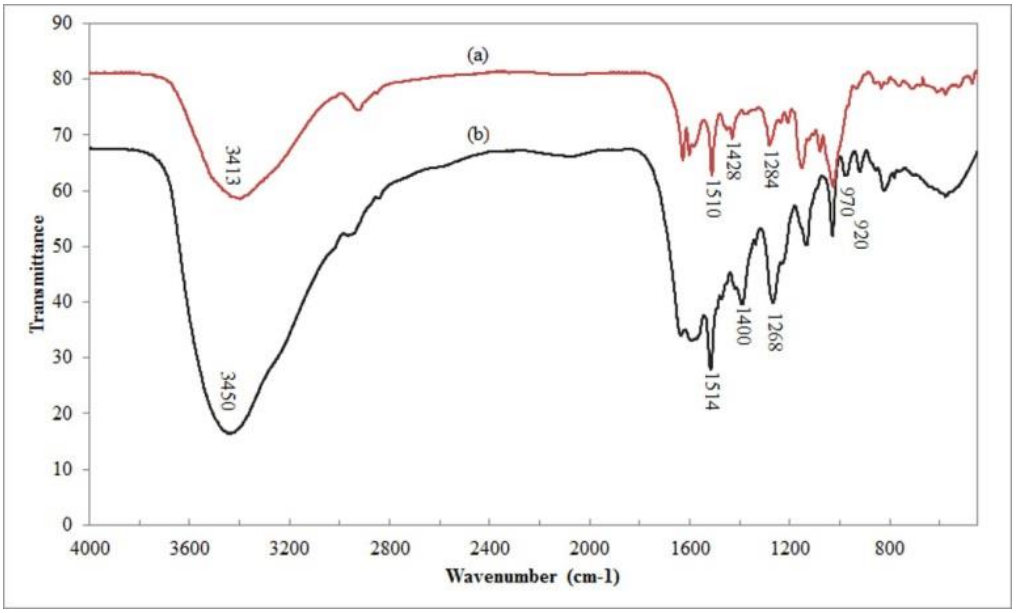


Figure 6. 4 FT-IR spectrum of curcumin (a) and CurGTMAC (b) measured in KBr pellets.

The spectra of curcumin and CurGTMAC are illustrated in Figure 6.4. To facilitate FT-IR spectra analysis, the chemical structure of CurGTMAC, proposed as a result of MS analysis, was used to compare major difference with the structure of curcumin reactant. In comparison, the major difference of these two compounds arose from the possession of N-$(CH_3)_3$, observed only in the structure of CurGTMAC. As expected, absorption bands related to R-N-$(CH_3)_3$ mentioned in literatures (Lin-Vien, 1991) were observed at 1000-900 cm^{-1} in the spectrum of the modified curcumin only. The FT-IR analysis result was corresponding with the MS analysis result. Therefore, the structure of CurGTMAC was insistent.

6.3.1.3 Thermogravimetric Analysis

To investigate changes in properties of CurGTMAC as a function of increasing temperature, thermogravimetric analysis (TGA) was conducted by individually placing approximately 5 mg of CurGTMAC and curcumin in a pan. The samples were then heated up from 80 to 800 ^{o}C at the heat flow rate of 10 ^{o}C/min in an atmosphere of nitrogen. The result was illustrated as a thermogram for the decomposition of both samples and presented in Figure 6.5.

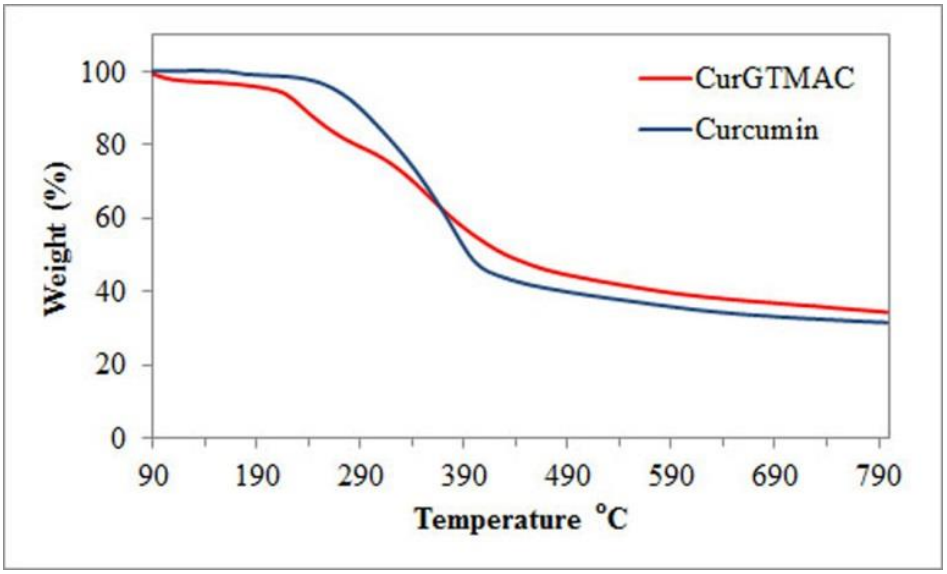


Figure 6. 5 Thermogram for decomposition of curcumin and CurGTMAC in an inert atmosphere.

From Figure 6.5, thermal curves of curcumin and CurGTMAC were slightly different indicating that both compounds had different thermal behavior with increasing temperature. A close observation of the curves revealed that curcumin underwent only one step degradation in the range of 210-300 ^{o}C with the weight loss of ~ 49 %. Contrarily, CurGTMAC showed the occurrence of two distinct steps of degradation and weight loss. The first degradation step was at the

temperature between 210 ^{o}C and 300 ^{o}C with a weight loss of ~ 17 % and the second degradation step was in the range of 300-437 ^{o}C with a weight loss of ~28 %. Two-step degradation was likely to specify that there were 2 different components of combustion products from CurGTMAC.

6.3.1.4 UV-visible Spectral Data and Calibration Curves

Powder of curcumin and CurGTMAC was dissolved in ethanol and the solvent mixture of ethanol and deionized water, respectively, and the absorbance values of the solutions were measured by means of UV-visible spectroscopy. Plots of absorbance values of curcumin and GTMAC-modified curcumin as a function of wavelengths in the range of 200-900 nm are illustrated in Figure 6.6.

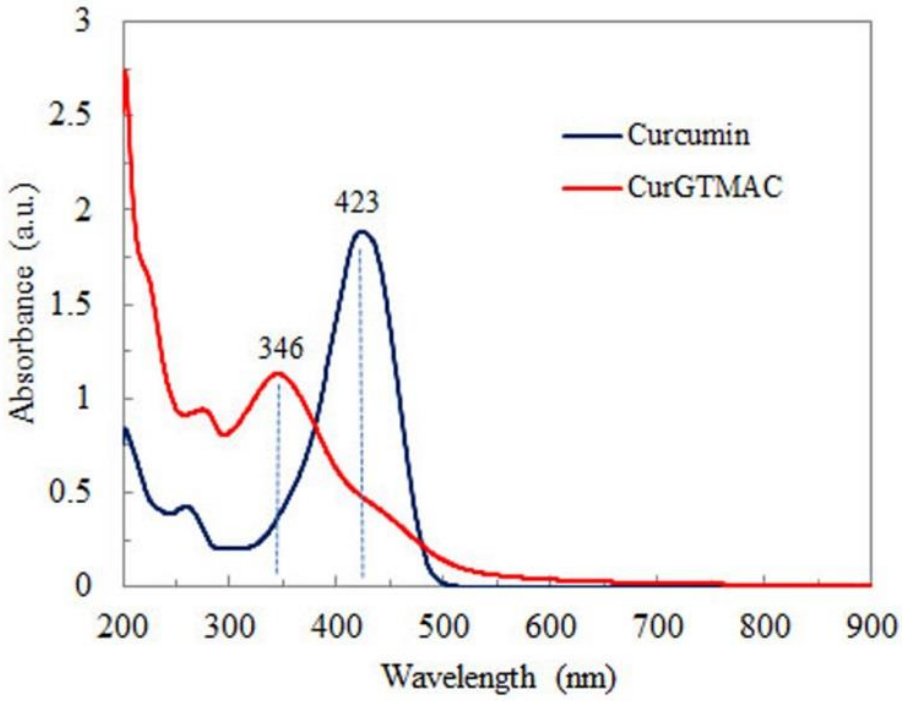
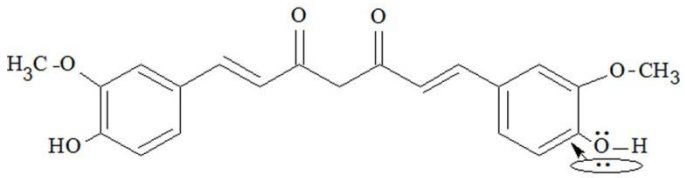


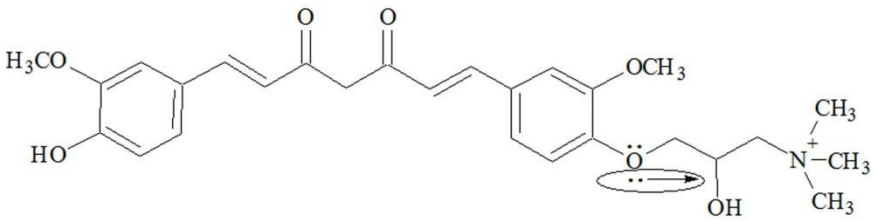
Figure 6. 6 UV-visible absorption spectra of modified curcumin (in 1:1 mixture of ethanol and deionized water) and curcumin (in ethanol).

As seen in Figure 6.6, the absorption spectrum of curcumin in ethanol displayed

2 distinct peaks at the wavelength range of 340-480 and 200-290 nm. The maximum absorption wavelength was at 423 nm. CurGTMAC also showed similar absorption characteristic to curcumin but the intense peak shifted significantly to near ultraviolet region. The maximum absorption wavelength of CurGTMAC was at 346 nm. The replacement of hydrogen atom on phenolic group by a quaternary ammonium group as seen in Figure 6.7 may explain the change of the spectral band of CurGTMAC compared to that of curcumin.



Curcumin



CurGTMAC

Figure 6. 7 Replacement of hydrogen atom at a phenolic group of curcumin by a quaternary ammonium moiety from glycidyltrimethylammonium chloride.

When the hydrogen atom on a phenolic group of curcumin is replaced by a quaternary ammonium group of GTMAC, the delocalization of lone pair electrons on the oxygen atom of the phenolic group to π-system of curcumin molecules would be affected since the quaternary ammonium moiety is a strong withdrawing group. Electrons will be pulled towards the atom, of which

electronegativity is higher or containing a positive charge. Therefore, size of conjugated system of curcumin may be reduced. The energy difference between HOMO and LUMO may be higher. Higher energy or shorter wavelength of electromagnetic radiation will be absorbed. The shifting of the absorption band from longer to shorter wavelength as seen in Figure 6.6 is called hypsochromic shift or blue shift.

With the peak absorption in UV region, the modified curcumin with a molar extinction coefficient (ε_{molar}) of 1,844 $M^{-1}cm^{-1}$ should be effective enough to function as a natural-based dye with good UV absorbing properties.

6.3.2 In Vitro Cytotoxicity Test

To be assured that CurGTMAC will not cause any health risk problems to human beings; its cytotoxicity was evaluated using MTT assay by assessing changes in mitochondria metabolic activity. The percentage of cell viability of normal human skin fibroblasts cells was assessed and the results were presented in Figures 6.8 and 6.9.

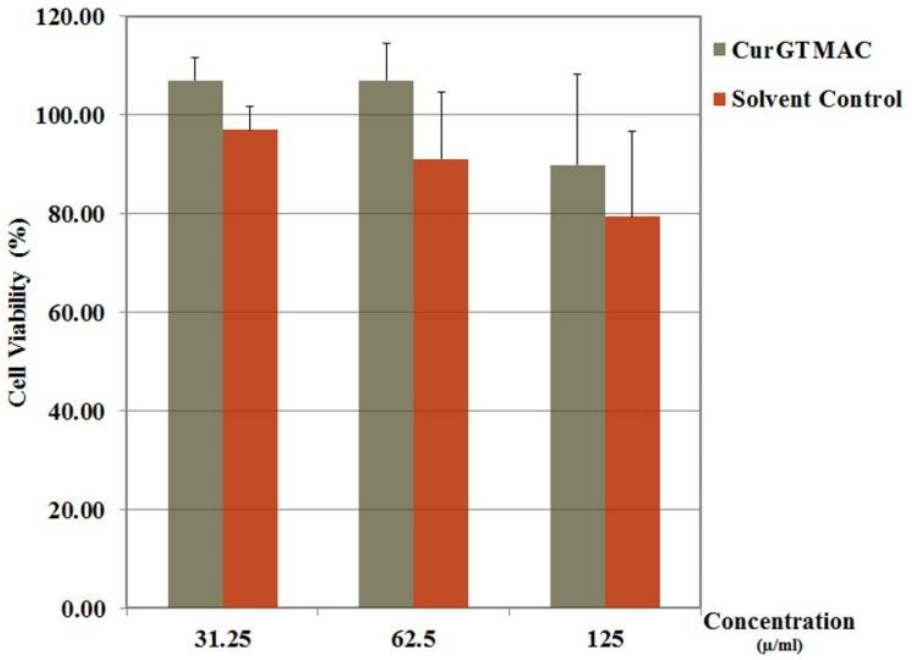


Figure 6. 8 Cytotoxicity of CurGTMAC to normal human skin fibroblasts cells after 24 hours exposure to the serial dilutions of CurGTMAC, determined by MTT assay.

As seen in Figure 6.8, CurGTMAC solution in the 1:1 solvent mixture of ethanol and deionized water induced less reduction in % cell viability at all given concentrations compared to solvent control cultures at the same concentration. At the concentration of 125 µg/ml of CurGTMAC solution, cell viability of normal human skin fibroblasts cells was reduced to 89.89 % after 24 hours of exposure to the solution. Ethanol is commonly known for its toxicity to cultured cells. It can penetrate to cell membrane and perturb the membrane structure and function and finally damage cell. However, an addition of ethanol was necessary since the water solubility of CurGTMAC was not sufficient possibly due to its comparatively hydrophobic structure with a limited number of quaternary ammonium groups.

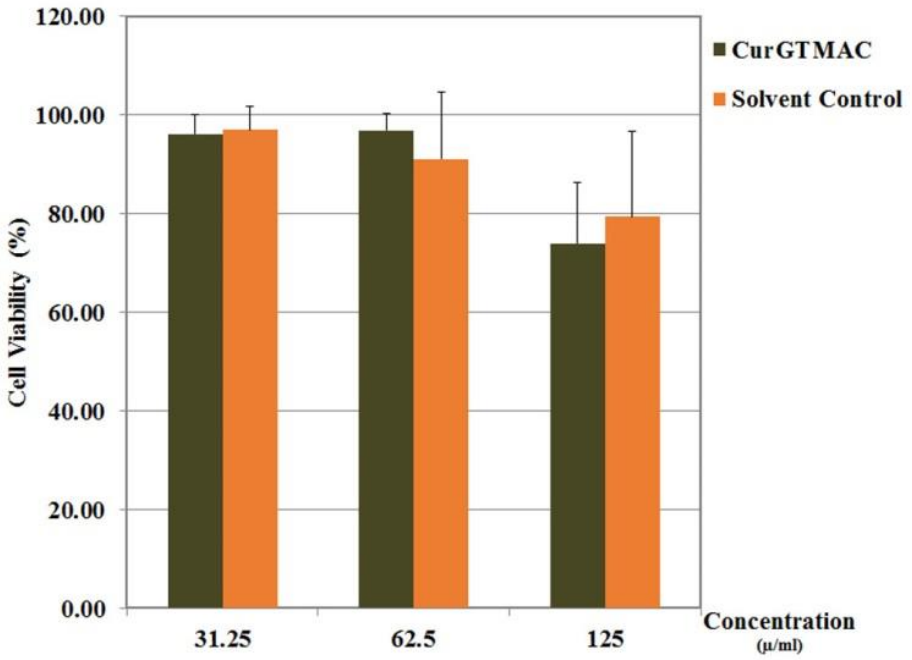


Figure 6. 9 Cytotoxicity of CurGTMAC to normal human skin fibroblasts cells after 48 hours exposure to the serial dilutions of CurGTMAC, determined by MTT assay.

After 48 hour exposure, the sensitivity of cultured cells to the solvent control was increased with an increase in concentration of the solvent control, whereas similar results were obtained from CurGTMAC at the concentrations of 31.25 and 62.5 µg/ml. % cell viability was slight decreased as seen in Figure 6.9. At the concentration of 125 µg/ml, however, the reduction in cell viability caused by both CurGTMAC and solvent control was more pronounced. It was possible that the reduction in cell viability caused by CurGTMAC dissolved in solvent mixture was significantly induced by ethanol. Therefore, the cytotoxicity of CurGTMAC itself was not significant.

6.3.3 Skin Irritation Test

From consumer perspective on safety, allergic problems to dyes and chemicals in textile products have been increasingly concerned. In this work, CurGTMAC was investigated for its irritant properties according to ISO 10993-10:2010. After a single application of the test substance to intact skin, albino rabbits were observed for any change of the appearance of their skin surface after 1, 24, 48, and 72 hours of the patch removal. The reactions, defined as erythema & eschar formation and oedema, were evaluated based on the scoring system of skin reaction shown in Table 6.1. The scores of the skin reaction in rabbit from monitoring erythema and eschar formation and oedema formation were presented in Tables 6.3 and 6.4, respectively.

Table 6. 3 Evaluation result of CurGTMAC on skin irritation in rabbit by monitoring erythema & eschar formation according to ISO 10993-10:2010 method.

Observation period	1 h				24 h				48 h				72 h			
Skin Application Site	Test		Ctrl		Test		Ctrl		Test		Ctrl		Test		Ctrl	
Relative Direction	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R
Rabbit Number																
1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 6. 4 Evaluation result of CurGTMAC on skin irritation in rabbit by monitoring oedema formation according to ISO 10993-10:2010 method.

Observation period	1 h				24 h				48 h				72 h			
Skin Application Site	Test		Ctrl		Test		Ctrl		Test		Ctrl		Test		Ctrl	
Relative Direction	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R
Rabbit Number																
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

The Primary Irritation Index (PII) of the test substance was 0.06. The PII less than 0.5 was considered negligible irritant. This study indicated that CurGTMAC could be used as a source of safe and effective modified natural dye which is non-irritant to the skin of rabbits.

6.4 Conclusions

An alternative approach was attempted to modify curcumin dye to be readily exploited and more in an applicable form. As a quaternary ammonium cationizing reagent commonly used to impart cationic moieties to many hydroxyl-bearing macromolecules, glycidyltrimethylammonium chloride was firstly introduced to alter the structure of curcumin. The reaction was considered environmental friendly since it was conducted in aqueous system with the presence of sodium carbonate as an alkaline. The functions of sodium carbonate were to make curcumin soluble and to open up the oxirane structure.

As characterized by MS, a novel curcumin-based compound was likely to possess one phenolic group and one quaternary ammonium cationic group. The maximum absorption wavelength of CurGTMAC was shifted closer to UV region. Cytotoxicity and skin irritation tests were brought to assure that the use of CurGTMAC could be safe to human beings. MTT assay was conducted to investigate its cytotoxicity to the normal human skin fibroblasts cells. No obvious sign of toxicity was indicated. For the skin irritation test, ISO 10993-10 was used to explore the potential of CurGTMAC to cause an allergy problem to the skin of a living species. The results showed no skin irritation on the skin of selected animal.

Chapter 7: Applications of GTMAC-modified Curcumin on cotton fabrics: Improving Durability of Functional Properties

7.1 Introduction

Curcumin is a typical natural yellow dye for textile coloration, but its use is rather limited to certain fibers, particularly cellulosic fibers due to its low affinity and poor water solubility properties. The chemical modification of curcumin had been previously conducted using glycidyltrimethylammonium chloride in an alkaline aqueous media. The major modified product was instrumentally characterized and identified as a quaternary ammonium cationized curcumin and simply named as CurGTMAC. Certain properties of CurGTMAC were investigated and reported in previous chapter of this thesis. To further explore, dyeability and other properties of CurGTMAC were needed to be investigated especially on cotton fiber.

Naturally, the affinity of cationic colorant to cotton fiber is problematic. To enhance this property, citric acid has been used to incorporate into the structure of cotton fiber before dyeing. In general, citric acid has been used to substitute formaldehyde releasing N-methylol compounds to impart durable press finishing properties on cotton fiber (Katovic, 2002). Its combination with sodium hypophosphite also could provide antibacterial and antifungal properties to cotton substrate (Budimir, *et al.*, 2012).

Citric acid has been beneficially used not only in textile application, but in others as well including tissue engineering (Yang, *et al.*, 2004) and clinical study (Nagoba, *et al.*, 1998), principally due to its non-toxicity, relatively inexpensive cost, and readily available resource, as well as friendly to the environment.

Moreover, citric acid has been used as a friendly crosslinking agent in a combination with positively charged silver/titanium dioxide nanocomposite in

acidic solution to introduce additional negative charged groups on wool fabric. This application successfully induced antibacterial activity to the wool substrate (Montazer, *et al.*, 2011). Another application of citric acid as a crosslinking agent was related to the utilization of *β*-cyclodextrin and thymol oil for durability of antibacterial activity against repeated washing. Citric acid was used to graft *β*-cyclodextrin onto cotton fabric in which monoterpene thymol oil was subsequently encapsulated. As a result, thymol oil could retain longer in the cavities of *β*-cyclodextrin moieties, adhered firmly to the structure of cotton fiber, and released gradually from the grafted fiber (Rukmani & Sundrarajan, 2012).

In this experiment, citric acid was selected to apply to cotton fabric mainly due to its relative ease of use and economic attractiveness. Strong anionic dye sites on cotton fabric were expected to be dominant and they were investigated by ATR-FTIR analysis to compare changes in functional groups with the untreated cotton. The treated fabric was then dyed with a given series of concentrations of CurGTMAC. Dyeability, antibacterial activity, UV protection and durable press finishing properties of the treated fabric were evaluated.

7.2 Experimental

7.2.1 Treatment of Cotton Fabric with CA Crosslinking Agent

Pad-Dry- Cure Method

Cotton fabrics were used as a substrate for the treatment with citric acid (CA) crosslinking agent and sodium hypophosphite (SHP) catalyst. The cotton samples were padded to approximately 80 % pick up in an aqueous solution comprising of 8 % CA and 6 % SHP at room temperature on a Werner Mathis 2 roll horizontal padder. The samples were dried at 80 ^{o}C for 1 min using Mathis Labdryer followed by curing at 180 ^{o}C for 1.5 min. After treated by citric acid, cotton samples were washed and tumble dried twice according to AATCC Test Method 124 before investigating for other properties and uses.

The treated fabric was analyzed for the existence of citric acid moiety using ATR-FTIR and compared with untreated cotton fabric. The ATR-FTIR spectra of CurGTMAC dyed (CA-treated) cotton, CA-treated cotton, and untreated cotton were illustrated in Figure 7.1. The treated fabric was also evaluated for its durable press properties and the results are presented in Table 7.1 and Figure 7.2.

7.2.2 Dyeing of CA-Crosslinked Cotton Fabric with CurGTMAC

CurGTMAC dye was dissolved in a 1:1 solvent mixture of ethanol and deionized water. Then the solution of CurGTMAC dye was applied to 1 gram of CA-treated cotton fabric with four different concentrations, i.e., 0.5, 1.0, 1.5 and 2.0 % on weight of fabric (o.w.f.) by exhaustion dyeing method. The dyeing was carried out in a water bath shaking dyeing machine with the material-to-liquor ratio of 1:15 at 70 ^{o}C for 45 minutes. The dyed fabrics subsequently called the

CurGTMAC-dyed fabrics were then rinsed thoroughly with deionized water and air dried. All CurGTMAC-dyed fabrics were measured for their color strength (*K/S*) and colorimetric values *L*a*b*C*h*. The *K/S* values were plotted as a function of concentrations of CurGTMAC and are presented in Figure 7.3. CIE *L*a*b*C*h* values of the dyed fabrics were compared to those of curcumin-dyed fabrics and all results are presented in Figure 7.4 and Table 7.2.

7.2.3 Evaluation of UV Protection Properties of CurGTMAC-dyed Fabrics and Its Durability

Sun Protective Clothing-Evaluation and Classification: Australian/New Zealand Standard AS/NZS 4399:1996

All samples were specifically determined for the rated ultraviolet protection factor at 4 different directions using a Varian Cary 300 Conc UV-Visible Spectrophotometer. Sample transmission data (%*T*) in the range of 280-400 nm were recorded. Mean of the ultraviolet protection factor (UPF) was calculated according to the method described in the Australian/New Zealand Standard AS/NZS 4399:1996. All data were recorded as before laundering. The equations used to calculate mean ultraviolet protection factor (UPF) are presented below.

$$\mathrm{UPF} = \frac{\sum_{290}^{400} \mathrm{E}(\lambda) \cdot \mathrm{S}(\lambda).\Delta\lambda}{\sum_{290}^{400} \mathrm{E}(\lambda) \cdot \mathrm{S}(\lambda) \cdot \mathrm{T}(\lambda).\Delta\lambda}$$

Where $\mathrm{E}(\lambda)$ = relative erythemal spectral effectiveness

$\mathrm{S}(\lambda)$ = solar spectral irradiance (W m^{-2} nm^{-1})

$\mathrm{T}(\lambda)$ = spectral transmittance of the fabric at wavelength λ

$\Delta(\lambda)$ = wavelength interval (nm)

$$\text{Mean UPF} = (UPF_1 + UPF_2 + \ldots + UPF_N)/ N$$

Where

N = number of specimens (AS/NZS 4399:1996).

Color fastness to Laundering: Accelerated-AATCC Test Method 61-2010

The CurGTMAC-dyed specimens were laundered according to AATCC test method 61-2010 using test no. 2A. Methodological details of the standard test method are described in Chapter 3 section 3.3.4.

After laundering, the CurGTMAC-dyed specimens were determined for the rated ultraviolet protection factor and the obtained results were numerically recorded as after laundering. To investigate the durability to laundering, the CurGTMAC-dyed specimens were repeatedly laundered for 10, 20 and 30 laundering cycles. Then the mean of UPF values of individual specimens before and after laundering was plotted as a function of concentrations of CurGTMAC and the results are illustrated in Figure 7.6.

7.2.4 Evaluation of Antibacterial Activity of Curcumin and CurGTMAC on Cotton Fabric

Four concentrations of curcumin and CurGTMAC dye solutions (25, 50, 75, and 100 ppm) were prepared using the 1:1 mixture of ethanol and deionized water as a solvent. The cotton fabrics were impregnated with each dye solution at room temperature and then padded to obtain a wet pick-up of 62-65 % on a Mathis 2 roll horizontal padder. Samples were dried at 80 ^{o}C for 3 minutes using Mathis Labdryer and washed with deionized water to remove the excess compound before re-drying. The dyed samples were evaluated for their antibacterial activity

against Gram-positive bacteria (*S. aureus*). The methodological details are described in Chapter 3 section 3.4.1 and 3.4.2.

7.3 Results and Discussion

7.3.1 Treatment of Cotton Fabric with CA Crosslinking Agent

7.3.1.1 ATR-FTIR of CA-treated Cotton

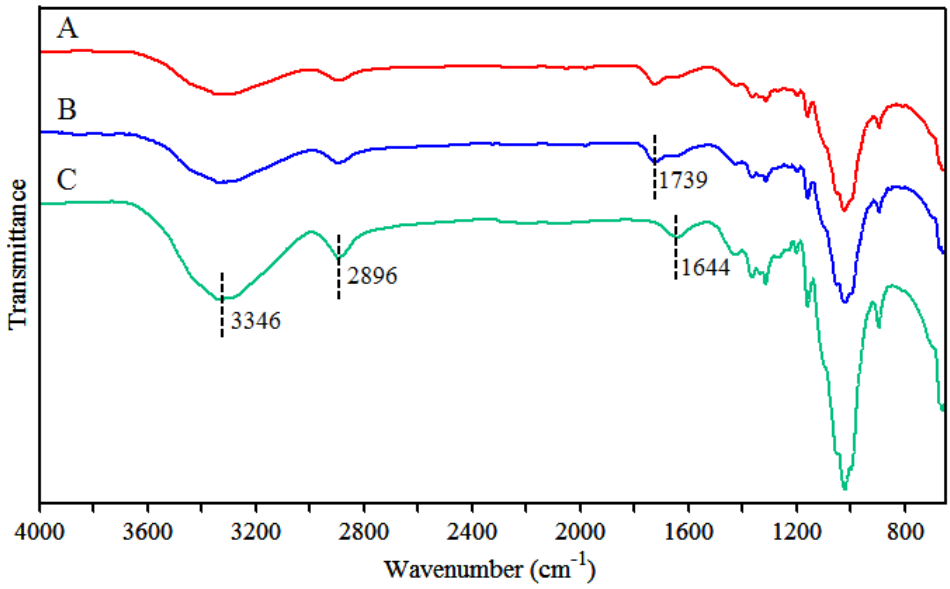


Figure 7. 1 ATR-FTIR spectra of (A) CurGTMAC dyed (CA-treated) cotton, (B) CA-treated cotton, and (C) untreated cotton.

From Figure 7.1, by comparison, most outstanding peaks of each spectrum appeared in similar regions of the infrared spectrum such as peaks at 3346 cm^{-1} and 2896 cm^{-1}. These two peaks revealed the stretching of free hydroxyl groups and methylene groups, respectively. The differences were mainly only at the wave number of 1644 cm^{-1} and 1739 cm^{-1}. The peak at 1644 cm^{-1} assigned to -OH bending disappeared for both treated fabrics and a new peak occurred at 1739 cm^{-1}. It was assigned to carbonyl group; thereby supporting the formation of ester carbonyl linkages between cellulose molecules and citric acid (Lewis & Voncina, 1997).

7.3.1.2 Durable Press Properties of CA-treated Cotton Fabric

After washed and tumble dried twice, the CA-treated cotton fabric was conditioned at 65 % RH and 21±1^{o}C for 24 hours before evaluating the following properties: wrinkle recovery angle (WRA), tearing strength (TS), tensile strength at break (BS), and durable press appearance rating (DP Rating). The following experimental test methods employed were employed in this study: wrinkle recovery angle (AATCC Test Method 66-2008), tearing strength (ASTM D-1424-2013) using Elmatear, and tensile strength (Grab Test) (ASTM-D-5034-2013) using Instron 4411 H3139. All tests were carried out on fabrics in both warp (W) and filling (F) directions and all results are presented in Table 7.1.

Table 7. 1 Physical properties of untreated cotton and CA-treated cotton fabric.

Sample	% CA+% SHP (% wet pick up)	DP Rating*	WRA* (W+F)	BS Retention (%)		TS Retention (%)		Whiteness Index	
				W	F	W	F	ASTM	CIE
Untreated cotton	-	1	143	- (301.0±5.6)	- (250.2±17.3)	- (5.5)	- (4.3)	112.2	111.4
CA-Treated cotton	8+6 (80 %)	2.5	225	64 (191.7±7.0)	70 (175.3±18.1)	56 (3.1)	6 (2.6)	88.9	92.4

* After 2 washing cycles

From the results presented in Table 7.1, both positive and negative effects on the properties of treated cotton induced by citric acid were obviously noticed. As expected, wrinkle recovery angle (WRA) and durable press appearance rating (DP Rating) of treated cotton were significantly improved, whilst its tearing strength (TS) retention, tensile strength (BS) retention and whiteness index decreased. The reason for the reduction of the strength of the fabric especially tearing strength could be attributed to acid damage, scorching heat from long time exposure to high heat during curing process, and the restriction of stress distribution within the fibers (Kittinaovarut, 1998). After crosslinked, cellulose chains lost their flexibility. The distribution of an external force applied in a particular way to other molecular chains was rather inhibited. Moreover, the acidity of citric acid itself could also be a cause of the strength reduction. A decrease in whiteness index of the CA-treated fabric, generally influenced by polycarboxylic acids, could be a result of partial dehydration of CA and the formation of aconitic acid causing yellowing effect to the fabric (Ibrahim, *et al.*, 2002). The improvement of durable press appearance rating of CA-treated fabric is illustrated in Figure 7.2.

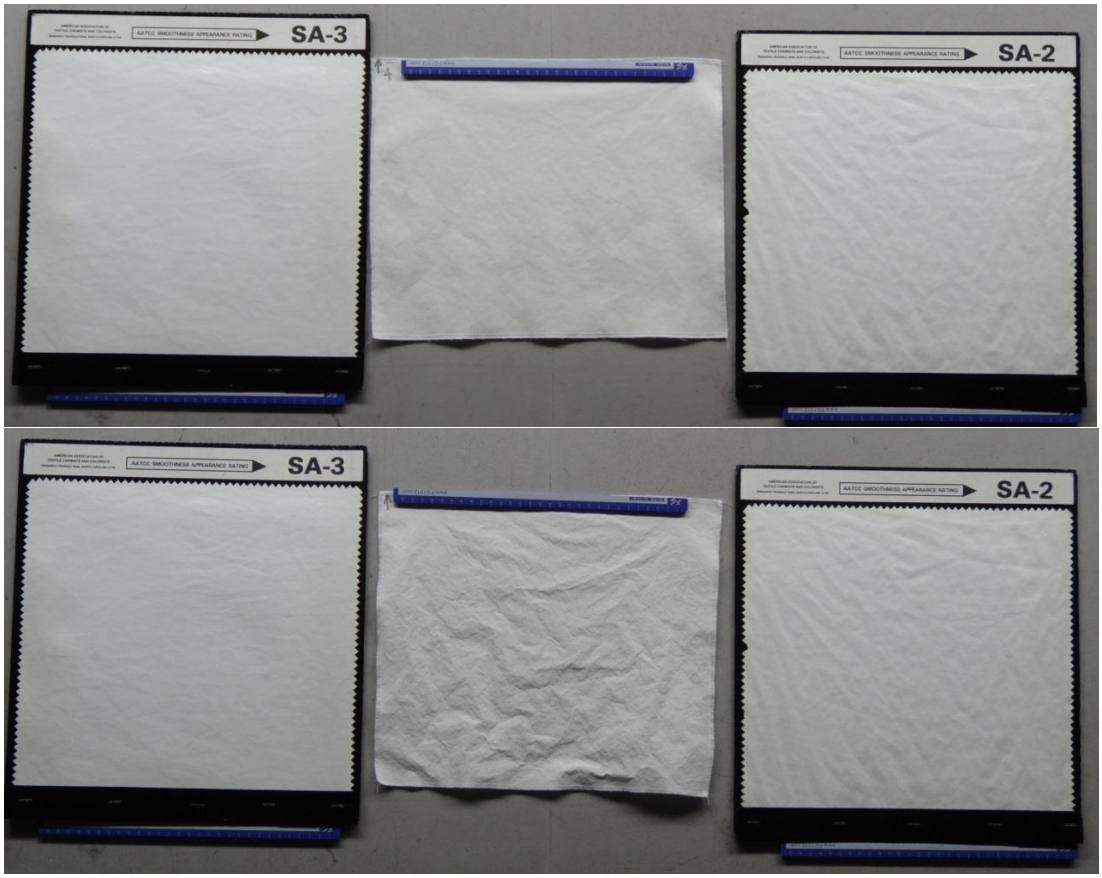


Figure 7. 2 Smoothness appearances of control fabric (middle, bottom) and CA-treated fabric (middle top).

7.3.2 Dyeing of CurGTMAC on the CA-Treated Cotton

The dyeability evaluation of CurGTMAC on the CA-treated fabrics was spectrophotometrically assessed by measuring their color strength (*K/S*). The *K/S* values obtained at 400 nm were plotted against concentrations of CurGTMAC dye solution as shown in Figure 7.3.

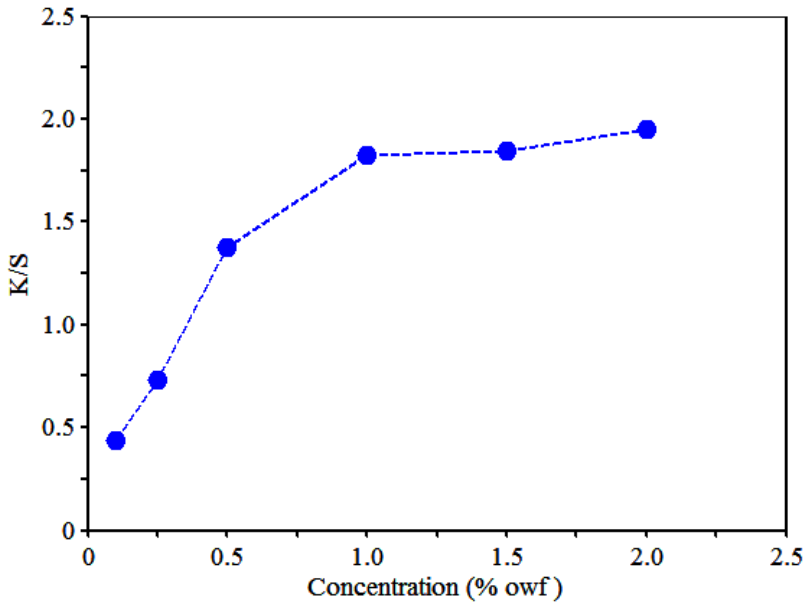


Figure 7. 3 Color strength at 400 nm of CurGTMAC-dyed cotton.

From the graph depicted above, the color strength of the CurGTMAC-dyed cotton fabric increased with increasing the dye concentration. It tended to reach a saturation point at the dye concentration above 1 % o.w.f., possibly due to the limitation of dye sites available on CA-treated cotton fabric as proposed in Figure 7.7.

The color shade of CurGTMAC on CA-treated cotton was a bright yellow, similar to that of curcumin-dyed cotton. However, their colorimetric properties were quite different. At a concentration, the blue-yellow coordinate (b^*) and color saturation (C^*) values of the CurGTMAC-dyed fabric were smaller as seen in Figure 7.4 and Table 7.2, respectively.

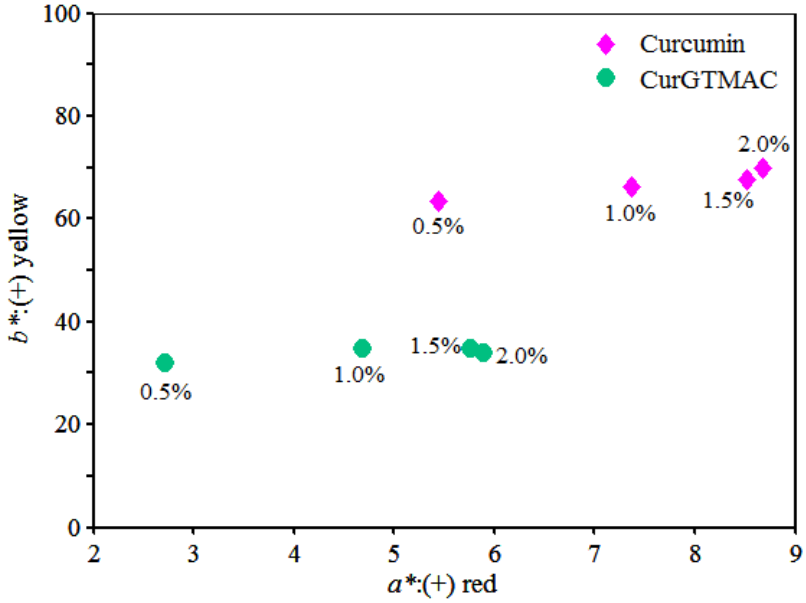


Figure 7. 4 *a*b** plot of curcumin dyed cotton and CurGTMAC dyed cotton at a given series of dye concentrations.

Table 7. 2 *L* C* h* of curcumin-dyed cotton (C-DC) and CurGTMAC-dyed (treated cotton) (CurG-DC).

Dye Conc. (% o.w.f.)	*L**		*C**		*h*	
	C-DC	CurG-DC	C-DC	CurG-DC	C-DC	CurG-DC
0.5	84.3	84.4	63.6	32.2	85.1	85.2
1.0	83.0	81.6	66.7	35.2	83.7	82.4
1.5	82.2	80.4	68.3	35.2	82.8	80.6
2.0	81.8	80.3	70.5	34.5	82.9	80.2

*L**: Lightness; *C**: Chroma; *h*: Hue.

7.3.3 Evaluation of UV Protection Properties of CurGTMAC-Dyed Fabric

The spectral transmittance measurement of all CurGTMAC-dyed fabrics was conducted in the wavelength range of 280-400 nm and the results are presented as plots of % transmission versus wavelength in Figure 7.5. The spectral transmittance of the samples was used to calculate mean UVA transmittance, mean UVB transmittance, and mean UPF according to equation from AS/NZS 4399:1996 and the numerical results are shown in Table 7.3.

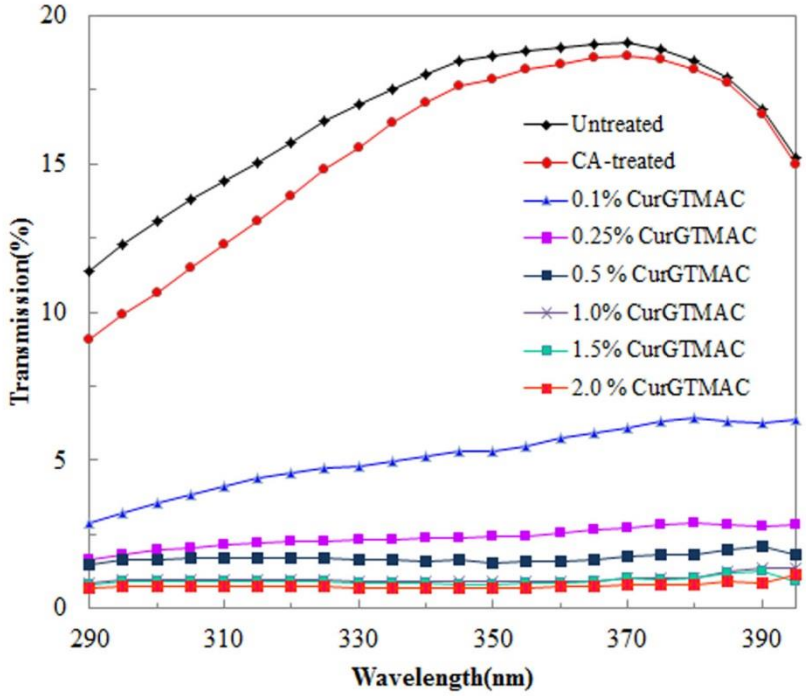


Figure 7. 5 Spectral transmission data of untreated cotton, CA-treated cotton and CurGTMAC-dyed cotton.

Table 7. 3 UV-protection properties of untreated cotton and CA-treated cotton before and after dyeing with CurGTMAC at 4 different concentrations

	Untreated cotton	CA-treated cotton	CurGTMAC-dyed cotton			
			Concentration of CurGTMAC (% o.w.f.)			
			0.5	1.0	1.5	2.0
mean UPF	6.91	8.03	60.18	102.92	109.99	133.29
Mean UVA transmission (%)	17.14	16.55	1.69	0.94	0.92	0.72
mean UVB transmission (%)	13.30	11.08	1.64	0.96	0.90	0.75

It is obviously seen from Figure 7.5 that % UV transmission of CA-treated fabrics dyed with CurGTMAC could be effectively reduced even when dyeing at a low concentration of CurGTMAC (0.1 % o.w.f.) and it was decreased with an increase in CurGTMAC concentrations up to 1 % o.w.f. Above this concentration, the reduction in % UV transmission was insignificant. These results were in the same direction with the color strength and mean UPF values of the fabrics indicated in Figure 7.3 and Table 7.3, respectively.

According to AS/NZS 4399:1996, both untreated cotton fabric and CA-treated cotton fabric with numerical UPF rating less than 15 were categorized under the non-rateable rating. Contrarily, the UPF values of all CurGTMAC-dyed fabrics were higher than 40. These fabrics were, therefore, classified as excellent UVR protection.

7.3.4 Durability to Home Laundering of UV-protection Properties of the CurGTMAC-Dyed Fabric

After laundering at 49 ^{o}C for 45 min in the aqueous solution of standard detergent without optical brightener and without chlorine, the spectral UV transmittance of CurGTMAC-dyed cotton fabrics was measured. Their UV transmission data and mean UPF values calculated according to AS/NZS 4399:1996 were obtained and recorded. The results are graphically presented in Figure 7.6.

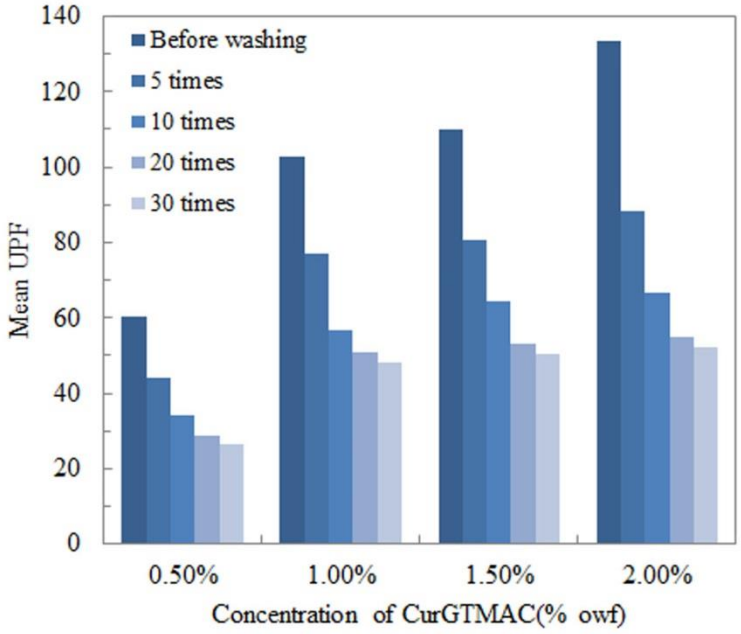


Figure 7. 6 Effects of repeated home laundering cycles to mean UPF of CurGTMAC -dyed cotton fabrics.

UPF values of all fabrics after repeated laundering for 30 cycles were still higher than 40 except for values obtained from the concentration of 0.5 % o.w.f. These fabrics could remain under excellent UV protection category after luandering. The durability toward the accelerated laundering test was likely due to an ionic bond between a negative charge of CA-treated cotton and a positive charge of quaternary ammonium group of the modified curcumin as proposed in Figure 7.7.

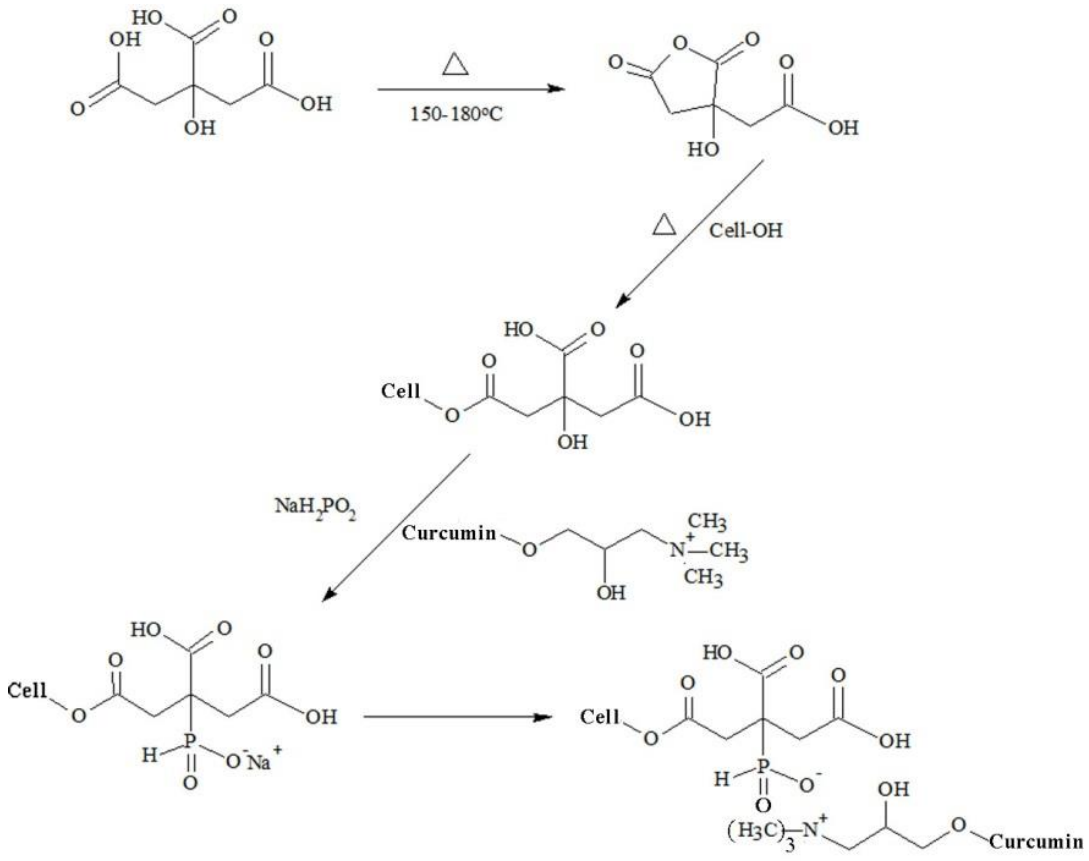


Figure 7. 7 Proposed reaction mechanism of cellulose initially crosslinked with citric acid in the presence of sodium hypophosphite and subsequently bonded with CurGTMAC.

The color fastness to laundering of CurGTMAC-dyed cotton fabric was also investigated. The chosen fabrics were CA-treated cotton fabrics individually dyed with CurGTMAC and curcumin as well as untreated cotton fabric dyed with curcumin. All fabrics were dyed with the concentration of 2.0 % o.w.f. Their color strength and CIE *L*a*b** Color difference before and after laundering for 10 cycles were measured and compared. All results are presented in Table 7.4.

Table 7. 4 *K/S* values at maximum wavelength of 3 different types of dyed cotton fabrics before and after repeated washing for 10 cycles according to AATCC 61-2010 no 2A and their CIE *L*a*b** color difference (D65/10^{o}).

Fabric Type	*K/S*	Color difference (ΔE)
CA-treated cotton dyed with CurGTMAC		
before repeated washing	2.736	12.143
after repeated washing	1.452	
CA-treated cotton dyed with curcumin		
before repeated washing	3.297	35.143
after repeated washing	0.549	
untreated cotton dyed with curcumin		
before repeated washing	8.116	45.811
after repeated washing	0.743	

As seen in Table 7.4, the difference of *K/S* values before and after laundering of all samples corresponded to their CIE *L*a*b** color difference values. The smallest color change was observed at CA-treated cotton fabric dyed with CurGTMAC. It was indicated that the color fastness to laundering was improved for cotton fabric initially treated with citric acid prior to dyeing with CurGTMAC.

7.3.5 Evaluation of Antibacterial Activity of Curcumin and CurGTMAC on Cotton Fabric

According to the literature review mentioned previously, curcumin exhibited its antibacterial activity both in solution and after applied to textile substrates mainly cotton and wool fibers (Han & Yang, 2005; Reddy, *et al.*, 2013). The durability to

washing of curcumin on cotton substrate was comparatively poor compared to wool substrate. In this experiment, antibacterial activity of the modified curcumin or CurGTMAC was evaluated and compared with that of curcumin according to ASTM E 2149 and AATCC 100-2010. The methodological details of both standard test methods were described in Chapter 3.

Since CurGTMAC was not completely purified, each concentration of CurGTMAC solution was needed to be interpolated so that the comparison with curcumin concentration could be made more efficiently. Accordingly, the relationship between dye concentration and its absorbance at 346 nm was established for both curcumin and CurGTMAC dyes as shown in Figure 7.8.

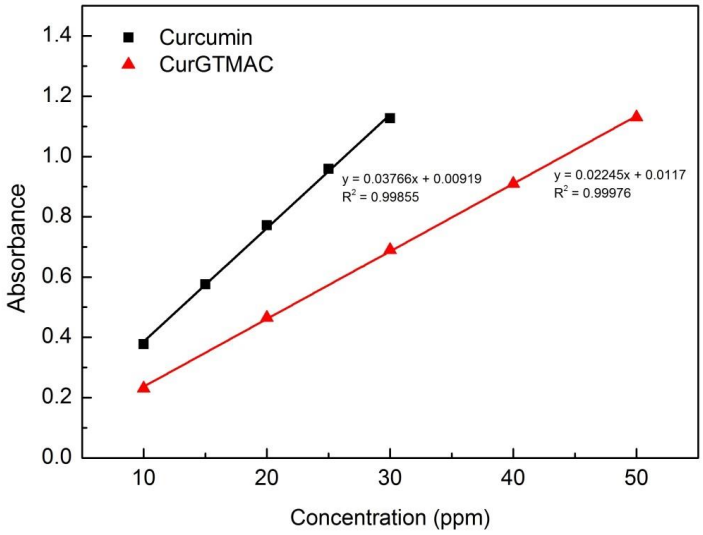


Figure 7. 8 Calibration curves between dye concentration and its absorbance of curcumin and CurGTMAC dyes.

From Figure 7.8, the linear regression analysis of the calibration curves of both compounds led to a linear regression equation of $Y = 0.03766X + 0.00919$, $R^2 = 0.99855$ for curcumin and $Y = 0.02245X + 0.0177$, $R^2 = 0.99976$ for CurGTMAC. Based on these two equations, concentrations of curcumin corresponding to an equivalent absorbance of CurGTMAC were calculated and used in preparing the substrates for antibacterial activity evaluation.

Cotton substrates dyed with curcumin and CurGTMAC, which exhibited equivalent absorbance, were evaluated for their antibacterial activity against *S.aureus* according to ASTM E2149. The experiment was conducted at the Microbiology Department of Chulalongkorn University in Thailand and results are presented and compared in Table 7.5.

Table 7. 5 Assessment of antibacterial activity against *S. aureus* of curcumin and CurGTMAC dyed cotton fabrics: ASTM E2149.

Samples	Visual Assessment & Reduction in CFU (%)	
Dye Concentration	Curcumin	CurGTMAC
25 ppm		
	51.85	64.44
50 ppm		
	62.96	62.96
75 ppm		
	62.96	69.25
100 ppm		
	74.07	82.96

As seen in Table 7.5, cotton fabrics dyed with either curcumin or CurGTMAC were active against *S.aureus*. CurGTMAC on cotton substrate exhibited a slightly higher pronounced reduction in population of microorganism tested than curcumin on the same substrate. However, the maximum values of % reduction were only about 74

for curcumin and about 83 for CurGTMAC indicating that both compounds were not potent enough to be classified as bactericidal compound (Kim, *et al.*, 2014; Nishanth, *et al.*, 2014). They belonged to the category of bacteriostatic agents since they could function only to prevent the growth and reproduction of bacteria, but not to kill them.

After the antibacterial activity against Gram-positive bacteria of curcumin and CurGTMAC on cotton substrate was evaluated, the experiment against Gram-negative bacteria was likewise conducted. However, the service at the previous institute conducted for Gram-positive is no longer available. The investigation on the antibacterial activity against Gram-negative bacteria was accomplished at The Hong Kong Polytechnic University and National Nanotechnology Center Thailand according to AATCC 100-2010. The methodological details were described in Chapter 3. Untreated cotton was individually dyed with 2 % o.w.f. of curcumin and CurGTAMC and was tested for the antibacterial activity against Gram-negative bacteria, *E.coli*, and the results are presented in Table 7.6.

Table 7. 6 Evaluation of antibacterial activity against *E.coli* of curcumin and CurGTMAC dyed cotton fabrics: AATCC 100-2010.

Fabric Samples	Reduction in CFU (%)
Curcumin on Cotton	31.38
CurGTMAC on Cotton	86.77

From % reduction of bacteria shown in Table 7.6, both samples were found active against Gram-negative bacteria, *E.coli*, and still belonged to bacteriostatic group since the % reduction was much lower than 99 %.

Antibacterial activity of curcumin against both types of bacteria is derived from an important contribution of phenolic group. It can partition cytoplasmic membranes of bacteria causing leakage of cell constituents. Function of membrane is, therefore, disrupted and subsequently caused cell death (Campos, *et al.*, 2009; Van Schie & Young, 2000).

In term of CurGTMAC, its functional groups as characterized by MS were a phenolic group with a quaternary ammonium cationic group. Mode of antibacterial action of CurGTMAC should, therefore, from both phenolic and quaternary ammonium groups. Principally, quaternary ammonium compounds function similarly to phenolic compounds. The positive charges of the compound can exert a strong electrostatic interaction with negative sites found on the membrane surface of bacteria causing cell to lose important constituents and finally cell lysis.

As for CurGTMAC, its synthesis and application is first time reported herein, therefore, only antibacterial results of curcumin as a bacteriostatic compound against both Gram-positive and Gram-negative bacteria selected were in accordance with other publication results (Na, *et al.*, 2011; Preedy, 2014; Zhu, 1998). The synergistic effects of curcumin with other compounds mainly antibiotics were also reported. The combination of curcumin with antibiotics led to improve its efficiency against bacteria (Betts & Wareham, 2014; Mun, *et al.*, 2013; Na, *et al.*, 2011; Nishanth, *et al.*, 2014b).

In this experiment, citric acid was used to crosslink cotton substrate before the application of CurGTMAC. In general, organic acids have been used for many years as antimicrobials especially in food industry (In, *et al.*, 2013; Mani-López, *et al.*, 2012). They can lower internal pH of microorganism cells resulting in unfavorable conditions for cells growth or multiplication. Cell membrane permeability is also affected by acids including citric acid (Beuchat, 1998; Topley & W., 1983). It is effective against microorganisms equivalently to quaternary ammonium chloride products widely used as commercial antibacterial finishing agents, when applied to cotton fabric with the presence of phosphoric acid catalyst (Vukusic, *et al.*, 2009). Accordingly, the antibacterial activity of CA-treated cotton and CurGTMAC on CA-treated cotton was evaluated against *E.coli* and the results are shown in Table 7.7.

Table 7. 7 Evaluation of antibacterial activity against *E.coli* of CA-treated cotton and CurGTMAC on CA-treated cotton*:* AATCC 100-2010.

Fabric Samples	Reduction in CFU (%)
CA-treated cotton	99.80
CurGTMAC on CA-treated cotton	99.99

From the results above, citric acid treated on cotton fabric was also effective against *E. coli*. This effect may be from the contribution of both free carboxyl and carboxylate groups of citric acid, available after crosslinking the cellulose chains in cotton fabric. Free carboxylic acids normally release hydroxonium ions after dissociating in water to affect the survival of many kinds of bacteria (Kim, *et al.*, 2003). Therefore, when the esterified cotton fabric had a contact with the negative charges of cytoplasmic membrane of microorganism, transportation of cell constituent could be disrupted and caused damages to cell membrane (Vukušić, *et al.*, 2011). Free carboxylic acids also could interact with sulfur-containing protein in certain types of bacteria and subsequently kill them (Mithil Kumar, *et al.*, 2012). The result in Table 7.7 showed the synergistic effect of curcumin and citric acid. The highest % reduction of bacteria, *E.coli,* was achieved on CA-treated cotton both before and after dyeing with CurGTMAC.

7.4 Conclusions

The application of citric acid with sodium hypophosphite provided certain

advantages to cotton substrate. Firstly, durable press properties were achieved as expected. Secondly, CurGTMAC dyeing process on cotton was quite simple. It could be conducted at the temperature of 70 ^{o}C without auxiliaries. Finally, the synergistic effects in antibacterial activity against *E. coli* were observed from the combination use of citric acid and CurGTMAC on cotton substrate.

When cotton was dyed with CurGTMAC, the color shade of CA-treated cotton was relative bright yellow with the color strength of 1.95 at the dye concentration of 2.0 % o.w.f. The attractive force between CA-treated cotton fabric and CurGTMAC was possibly due to electrostatic interaction occurring between positive charges of CurGTMAC and negative charges from free carboxyl and carboxylate groups of CA-treated cotton fabric developed by the esterification reaction between functional groups of cotton and carboxylic groups of citric acid. Moreover, the treated cotton fabric dyed with CurGTMAC at the concentration of 0.5 % o.w.f. acquired UPF rating higher than 40 indicating possessing excellent UV protection properties according to AS/NZS 4399:1996. The fabric could effectively block out solar ultraviolet radiation. After accelerated laundering, the CurGTMAC-dyed fabric exhibited good durability. This result strongly convinced the possibility of the existence of the electrostatic interaction between fibers and CurGTMAC molecules. Another important finding from this experiment was the antibacterial activity of curcumin and CurGTMAC on cotton. CurGTMAC-dyed cotton exhibited higher antibacterial activity against both Gram-positive, *S. aureus*, and Gram-negative, *E. coli*, than curcumin-dyed cotton. However, both curcumin and CurGTMAC-dyed cotton were still categorized under bacteriostatic group due to having % reduction

lower than 99.

Chapter 8: Conclusions and Recommendations

8.1 Conclusions

Curcumin, well recognized as a natural colorant with various biological properties, was chosen to investigate how to enhance the efficiency and widen their applications in textile industry. To emphasize the beneficial applications of curcumin, the research herein began with the extraction process of the volatile oil and curcumin from turmeric raw material. Then the curcumin colorant was applied to textile substrate to investigate its dyeability and other properties such as antibacterial activity. Finally, curcumin was used as a platform chemical to develop its chemical structure to enhance its technical properties and performances.

In the first experiment, dry turmeric powder was used to extract useful components by steam distillation method. The distillate coming out from the extraction was a milky pale yellow liquid. It was characterized for its components using gas chromatography-mass spectrometry (GC-MS). Curcumene and ar-turmerone were identified as major compounds detected from the extract which were in consistence with other data in literature. The turmeric residue from steam distillation was further isolated using ethanol at room temperature. A yellow substance which was characterized and subsequently identified by FT-IR as a curcumin colorant was obtained. Then, curcumin was dyed on cotton and three following synthetic fibers: poly(lactic acid), polyester and acrylic. The color measurements were conducted with the Macbeth CE-7000A spectrophotometer. The color of all dyed samples was yellow but their shade and lightness (L^*) were different. Dyed cotton had similar shade to dyed poly(lactic acid) but different from dyed polyester and acrylic

substrates. This observation was in consistence with *a** and *b** color coordinates. They were plotted in one quadrant for cotton and poly(lactic acid) substartes, but in another quadrant for polyester and acrylic substrates. The color fastness to laundering was conducted according to AATCC 61:2010. It was surprisingly found that curcumin could provide satisfactory fastness to laundering when dyed on polyester and acrylic fibers.

The second experiment was conducted to enhance the efficiency and widen the applications of curcumin in textile wet processing. The chemical modification of curcumin was initially performed by introducing N-phthaloylglycine and N-phthalylglycyl chloride to the backbone structure of curcumin. The purpose of this modification was to improve water solubility, dyeability, and antibacterial activity of curcumin. The modified product from the reaction of curcumin with N-phthalylglycine chloride was comparatively easy to purify than the product obtained from the reaction of curcumin with N-phthaloylglycine and it was instrumentally analyzed by ESI-MS. It was identified as a monoester curcumin with *m/z* 556. With the presence of N-phthaloyl protecting group, the structure of monoester curcumin was relatively more hydrophobic in nature compared to that of curcumin. Accordingly, an effort in dyeing on polyester fibers at high temperature was made. The color shade obtained from dyeing of monoester curcumin on polyester fabric was yellow similar to the color obtained from dyeing with curcumin but lighter in appearance with more negative value of *a** (greenness) and less positive value of *b** (yellowness). The dyed fabric was evaluated for antibacterial activity. It was found that the antibacterial activity of monoester curcumin dyed on polyester fabric was

lower than that of curcumin-dyed fabric possibly due to the contribution of the disappearance of a phenolic group of curcumin derivative.

With the stringent conditions to react curcumin with N-phthalylglycyl chloride, glycidyltrimethylammonium chloride (GTMAC) was replaced. This experiment was aimed to modify curcumin with GTMAC in an alkaline aqueous media. The product of this reaction was precipitated out of the solution with the addition of absolute ethanol, a short chain alcohol. The GTMAC-modified curcumin was characterized by liquid chromatography-mass spectrometry. From mass spectrum, the peak detected at m/z 520 was assigned as the compound of interest. It was a modified curcumin in which one phenolic group of curcumin was substituted by GTMAC moiety. This modified product was called as CurGTMAC. It was partially soluble in water and could become completely soluble in the 1:1 solvent mixture of ethanol and deionized water. The CurGTMAC solution was investigated for its absorbance characteristics. From the absorption spectrum of CurGTMAC in solution, hypsochromic shift to near ultraviolet region was observed. To ensure safety in product utilization, in vitro cytotoxicity and skin irritation test were conducted according to MTT assay and ISO 10993-10:2010, respectively. The results of in vitro cytotoxicity to normal human skin fibroblasts cells were not obvious at the concentration up to 125 μg/ml. The skin irritation result was negligible to the skin of albino rabbit tested.

To strengthen the fixation of CurGTMAC to cotton fabric, citric acid was incorporated as a crosslinking agent to impart negatively charged free carboxyl and

carboxylate groups to the backbone structure of cotton in the presence of sodium hypophosphite as a catalyst. The CA-crosslinked cotton fabrics were then dyed with CurGTMAC solution at the temperature of 70 ^{o}C in the absence of auxiliaries.

The color of dyed fabrics was yellow with the value of lightness parameter (*L**) higher than 80, similar to the lightness value of curcumin-dyed cotton but saturation value (*C**) was smaller and *a** value was lower. The color strength or *K/S* value of CA-crosslinked cotton fabric dyed with CurGTMAC at the concentration of 2 % o.w.f. was 1.95 at 400 nm as the maximum wavelength.

Finally, the experiments related to the applications of CurGTMAC were carried out. According to the result of hypsochromic shift phenomenon to near UV region with a high molar extinction coefficient (ε_{molar}) of 1,844 $M^{-1}cm^{-1}$, CA-crosslinked cotton dyed with CurGTMAC was investigated for the enhancement of UV absorbing properties. It was revealed that UPF rating of the crosslinked cotton fabric dyed with 1 % o.w.f. of CurGTMC was above 100 according to AS/NZS 4399:1996. After laundering using AATCC 61-2010 test condition no.2a, UPF rating was still higher than 40 indicating the durability of UV-protection of dyed cotton fabric against accelerated laundering.

As well recognized, curcumin exhibits antibacterial activity both in solution and after applied to cotton and wool fibers. Accordingly, the evaluation of antibacterial activity was conducted against both Gram-positive (S.*aureus*) and Gram-negative (*E.coli*) bacteria. The results showed that both curcumin and CurGTMAC had this property but they functioned only as bacteriostatic not bactericidal dye. In

comparison, CurGTMAC was more effective in inhibiting the growth or reproduction of bacteria. % reduction of colony forming unit of CurGTMAC was higher.

8.2 Recommendations

Recommendations for further study of curcumin and related compounds are listed hereinafter without regard to priority.

1. The turmeric volatile oil also has antibacterial activity. It has been claimed that it exhibits bactericidal effect. Accordingly, microencapsulation of the volatile oil for functional finish of cotton textile should be attempted.

2. To be more practical for coloration industry, the combination of CurGTMAC with other cationic dyes should be conducted on CA-crosslinked cotton fabric.

3. The use of citric acid and its catalyst should be optimized to gain highest dyeability and durable press properties with the lowest loss of strength and whiteness.

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