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EXTRACTION AND CHARACTERIZATION OF LAC DYE FROM THAI STICK LAC AND DEVELOPMENT OF LAC DYEING ON SILK AND COTTON

Miss Montra Chairat

A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Doctor of Philosophy in Chemistry

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Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

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งานวิจัยนี้เป็นการศึกษาการสกัด วิเคราะห์หาองค์ประกอบของสีครั่งจากครั่งดิบของประเทศไทยรวมทั้งศึกษาจลนพลศาสตร์และอุณหพลศาสตร์ทางเคมีของการย้อมสีครั่งบนเส้นใยไหมและฝ้าย ในการทดลองได้นำวิธีใหม่สำหรับการสกัดสีครั่งมาใช้ 2 วิธี คือ วิธีการสกัดด้วยไมโครเวฟและอัลตราโซนิก จากผลการทดลองพบว่า การสกัดสีครั่งโดยวิธีไมโครเวฟและอัลตราโซนิกได้ร้อยละโดยน้ำหนักของกรดแลคคาอิกสูงกว่าที่ได้โดยการใช้วิธีสกัดด้วยน้ำร้อน กล่าวคือ ร้อยละโดยน้ำหนักของกรดแลคคาอิกที่สกัดได้โดยวิธีการใช้น้ำร้อน อัลตราโซนิกและไมโครเวฟ มีค่าเท่ากับ 4.84, 5.70 และ 6.12 ตามลำดับ การวิเคราะห์องค์ประกอบของสีครั่งโดยวิธีคอลัมน์โครมาโทกราฟีพบว่าองค์ประกอบหลักคือ กรดแลคคาอิก เอ โดยมีกรดแลคคาอิก บี และกรดแลคคาอิก ซี เป็นองค์ประกอบรอง ซึ่งผลดังกล่าวจะเด่นชัดมากเมื่อสกัดด้วยวิธีไมโครเวฟ นอกจากนี้ยังพบว่าร้อยละโดยน้ำหนักของกรดแลคคาอิก บี ที่สกัดได้ด้วยวิธีการใช้น้ำร้อนและวิธีอัลตราโซนิกมีค่าใกล้เคียงกัน

การศึกษาจลนพลศาสตร์และอุณหพลศาสตร์ของการดูดซับในการย้อมสีครั่งบนเส้นใยไหมและฝ้าย พบว่าความสามารถในการดูดซับสีครั่งบนเส้นใยไหมและฝ้ายขึ้นอยู่กับ พีเอช ความเข้มข้นเริ่มต้นของสารละลายสีย้อม อัตราส่วนของเส้นใยต่อปริมาณของสารละลายสีย้อม และอุณหภูมิอย่างมีนัยสำคัญ และยังพบว่าอัตราเริ่มต้นของการดูดซับสีบนเส้นใยไหมและฝ้ายก่อนเข้าสู่สมดุลเพิ่มขึ้นตามอุณหภูมิ สมการอัตราของการดูดซับสีครั่งบนเส้นใยไหมและฝ้ายที่พีเอช 3.0 จัดเป็นปฏิกิริยาอันดับสองเสมือน โดยมีพลังงานก่อกัมมันต์ (E_a) เท่ากับ 47.0 และ 42.0 กิโลจูลต่อโมล ตามลำดับ การศึกษาทางอุณหพลศาสตร์แสดงให้เห็นว่าการดูดซับสีครั่งบนเส้นใยไหมและฝ้ายที่พีเอช 3.0 เป็นการดูดซับแบบแลงเมียร์และฟรอยด์ลิช ค่าเอนทัลปีของการดูดซับสีครั่งบนเส้นใยไหมและฝ้ายมีค่าเท่ากับ -31.4 และ -4.88 กิโลจูลต่อโมล ตามลำดับ ค่าที่เป็นลบเล็กน้อยแสดงให้เห็นว่าการดูดซับสีครั่งบนเส้นใยฝ้ายเป็นกระบวนการดูดซับทางกายภาพเท่านั้น นอกจากนี้ได้หาค่าพลังงานอิสระของกิบส์ (ΔG^o) และค่าเอนโทรปีที่เปลี่ยนแปลงไป (ΔS^o) ของการดูดซับสีครั่งบนเส้นใยไหมและฝ้ายอีกด้วย การศึกษานี้ยังพบอีกว่าการเคลือบเส้นใยฝ้ายและไหมด้วยไคโทซานช่วยเพิ่มปริมาณการดูดซับสีครั่งบนเส้นใย และยังลดปริมาณของสีที่หลุดออก

จากเส้นใยหลังการย้อมเมื่อเปรียบเทียบกับเส้นใยที่ไม่ได้ผ่านการเคลือบด้วยไคโทซาน การศึกษาถึงผลของการเติมเกลือโซเดียมคลอไรด์ต่อการดูดซับและการหลุดออกของสีครั่งจากเส้นใยฝ้าย พบว่าเกลือโซเดียมคลอไรด์ช่วยเพิ่มความสามารถในการดูดซับสีครั่งบนเส้นใยฝ้ายเมื่อไม่มีการควบคุมพีเอชของสารละลายสีครั่ง อย่างไรก็ตามผลการศึกษาการหลุดออกของสีครั่งหลังย้อม พบว่าสีครั่งหลุดออกจากเส้นใยได้ง่าย แต่การเติมเกลือไม่มีผลต่อการดูดซับสีครั่งของเส้นใยฝ้าย เมื่อมีการควบคุมพีเอชของสารละลายสีครั่งที่พีเอช 2.5, 3.0 และ 3.5 การวิจัยครั้งนี้ยังได้ใช้เทคนิคยูวี-วิสิเบิล สเปกโทรสโกปีเพื่อศึกษาผลของพีเอชและผลของไอออนโลหะบางตัวที่มีต่อสีครั่งที่สกัดได้จากครั่งที่ได้จากต้นฉำฉาในภาคตะวันออกเฉียงเหนือของประเทศไทย โดยเปรียบเทียบผลการทดลองที่ได้กับสีครั่งที่ซื้อจากบริษัท Wako และสารมาตรฐานกรดแลคคาอิก เอ และกรดแลคคาอิก บี และได้เสนอแบบจำลองในการเกิดอันตรกิริยาระหว่างสารมาตรฐานกรดแลคคาอิก เอ และไอออนนิคเกิลขึ้นเป็นครั้งแรกอีกด้วย

สาขาวิชาเคมี ลายมือชื่อนักศึกษา……..…….

ปีการศึกษา 2547 ลายมือชื่ออาจารย์ที่ปรึกษา……..

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม……...

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม……...

MONTRA CHAIRAT : EXTRACTION AND CHARACTERIZATION OF LAC DYE FROM THAI STICK LAC AND DEVELOPMENT OF LAC DYEING ON SILK AND COTTON. THESIS ADVISOR : ASSOC. PROF. SAOWANEE RATTANAPHANI, Ph.D., 247 PP. ISBN 974-533-338-7

The extraction and characterization of lac dye from Thai stick lac and the adsorption kinetics and thermodynamics of lac dyeing onto silk and cotton fibres were carried out in this research project. Two new procedures for lac dye extraction from Thai stick lac were investigated. These procedures involved the ultrasonication and microwave-assisted extraction methods. It was found that the total percentage of laccaic acids obtained by the ultrasonication and microwave-assisted extraction methods was higher than that from the hot water extraction method. The total percentage of laccaic acid represented about 4.84, 5.70 and 6.12% by weight of lac dye extract by using the hot water, ultrasonication and microwave extraction methods respectively. The major component obtained from the three extraction methods by column chromatography was laccaic acid A with the minor laccaic acids being B and C, especially from the microwave extraction. In addition, the percentage of laccaic acid B obtained by the hot water and ultrasonication extraction methods were essentially the same.

The adsorption kinetic and thermodynamic studies of lac dyeing onto silk and cotton fibres indicated that the adsorption capacities are significantly affected by pH, the initial dye concentration, the material to liquor ratio (MLR) and temperature. The initial dye adsorption rates of lac dye on silk and cotton fibres before equilibrium time was reached increased at higher dyeing temperature. The pseudo second-order kinetic

model was indicated for both lac dyeing of silk and cotton fibres at pH 3.0 with activation energies (E_a) of 47.0 and 42.4 kJ/mol respectively. Also, batch equilibrium studies showed that the adsorption of lac dye on silk and cotton fibres at pH 3.0 was described by the Langmuir and Freundlich isotherms. The values of the enthalpy for the lac dyeing of silk and cotton fibres at pH 3.0 were -31.4 and -4.88 kJ/mol respectively. The small negative value of the enthalpy change (ΔH^o) suggested that the adsorption of lac dye on cotton was a physical process. Also, the free energy (ΔG^o) and entropy changes (ΔS^o) for the lac dyeing of silk and cotton fibres were determined. The pretreatment of the cotton and silk fibres with chitosan increased the dye adsorbed on the fibres and also decreased the dye desorbed from fibres compared to the untreated fibres. In addition, the effect of NaCl on adsorption and desorption of lac dyeing onto cotton was also studied. The results indicated that NaCl increased the adsorption ability of lac dye on cotton without pH control. However, the lac dye was then more easily desorbed from the cotton. Sodium chloride had no effect on the adsorption of lac dye on cotton at pH 2.5, 3.0 and 3.5. Furthermore, UV-VIS spectroscopic studies were carried out on the effect of pH and selected metal ions on Thai lac dye extracted from stick lac from the Rain tree in northeast Thailand. These results were compared with those from a commercial lac dye (Wako Company), and from laccaic acids A and B. The first model for the proposed interaction of laccaic acid A with nickel (II) ion was developed.

School of Chemistry

Academic Year 2004

Student's Signature……..

Advisor's Signature……..

Co-Advisor's Signature……....

Co-Advisor's Signature……

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Montra Chairat

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LIST OF ABBREVIATIONS

mL	Millitre
L	Litre
E:A:W	Ethyl acetate:Acetic acid:Water
M:A:W	Methanol:Acetic acid:Water
g	gram
Wt.	Weight
m/*z*	mass to charge value
HPLC	High Performance Liquid Chromatography
^{1}H-NMR	Proton –Nuclear Magnetic Resonance
^{13}C-NMR	Carbon-Nuclear Magnetic Resonance
MS	Mass Spectrometry
LRMS	Low Resolution Mass Spectroscopy
HRMS	High Resolution Mass Spectroscopy
ES	Electrospray
k_1	Rate constant of pseudo first-order adsorption
q_e	The amount of dye adsorbed per gram fibres at equilibrium
q_t	The amount of dye adsorbed per gram fibres at time *t*
k_2	Rate constant of pseudo second-order adsorption
h_i	The initial dye adsorption rate

LIST OF ABBREVIATIONS (CONTINUED)

A	The pre-exponential factor
E_a	Activation energy
R	Gas constant
$\Delta H^{\#}$	Enthalpy of activation
$\Delta S^{\#}$	Entropy of activation
$\Delta G^{\#}$	Free energy of activation
k_b	Boltzmann's constant
h	Planck's constant
T	Temperature (K)
Q	Adsorption capacity of the Langmuir isotherm
b	Langmuir constant
Q_f	Adsorption capacity of the Freundlich isotherm
ΔH^o	Enthalpy change
ΔS^o	Entropy change
ΔG^o	Gibbs free energy
K_c	Equilibrium constant
$C_{ad,e}$	Concentration of the dye adsorbed at equilibrium
C_e	Concentration of dye left in the dye bath at equilibrium
w/v	Weight by volume
v/v	Volume by volume
t	Time

LIST OF ABBREVIATIONS (CONTINUED)

C_o	Initial dye concentration
C_t	Concentration of dye after dyeing time *t*
V	Volume of dye solution
W	Weight of fibre used
MLR	Material to Liquor Ratio
mg/L	Milligram per Litre
R^2	Correlation coefficient
Temp	Temperature
min	minute
q_{de}	The amount of dye desorbed from the fibres
$\zeta_{plateau}$	Zeta-potential of the plateau
λ_{max}	Maximum wavelength
A_t	Absorbance of dye solution at time *t*
A_o	Absorbance of dye solution before dyeing

CHAPTER I

GENERAL INTRODUCTION

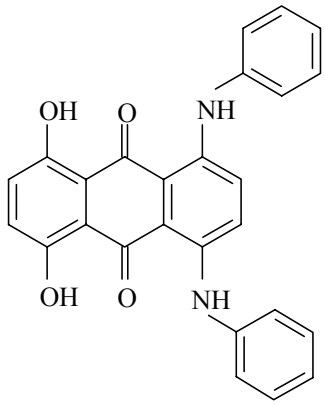
Dyes have been used since prehistoric time, for example in decorating bodies, in colouring the furs and skins that people wore and in paintings which adorned their cave dwellings (Christie, 2001). A dye is a substance which can be soluble in the dyeing medium during or at least during some stages of the dyeing process (Perkins, 1996). It can diffuse into fibres and interact to the molecules of the fibres by intermolecular or intramolecular forces which depend on the structure of dyes and the kind of fibres. Colourants can be obtained from nature sources and synthesis routes. They have been applied mainly to dye on textile materials such as protein and cellulosic fibres (Christie, Mather and Wardman, 2000). The following sections discuss the classification of synthetic dyes which are used in textile process.

1.1 Synthetic dyes

Synthetic dyes for textile fibres may be classified according to chemical structure or according to method of application (Perkins, 1996). The chemical classification and the application classification of colourants will be discussed separately in the next two sections.

1.1.1 Chemical classification

The classification of synthetic dyes according to chemical structure can be grouped into thirteen dye classes as shown in Table 1.1 (Perkins, 1996). Examples of the structures of anthraquinone dye, indigo dye and azo dye are illustrated in Figure 1.1.



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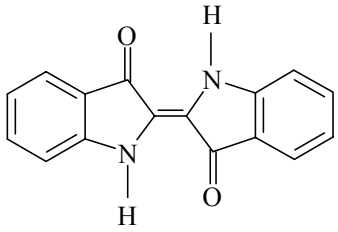
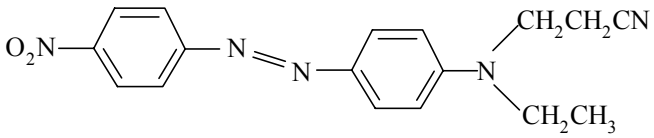


(b)



(c)

Figure 1.1 Examples of the structures of anthraquinone dye, indigo dye and azo dye. (a) C.I. Disperse Green 5, anthraquinone dye; (b) C.I. Vat Blue 1, indigo dye and (c) C.I. Disperse Orange 25, azo dye.

Table 1.1 Dyes by structural classes (Perkins, 1996)

Structural Class	Description	Application Classes
Azo dyes	Contain one or more azo, −N=N−, group	direct, azoic, reactive, acid, basic, disperse
Anthraquinone dyes	9,10-anthraquinone substituted at one or more of the four alpha positions (1,4,5 and 8)	vat, reactive, disperse, acid
Benzodifuranone dyes	Contain benzodifuranone (BDF) chromogen	disperse, (maybe others)
Polycyclic aromatic carbonyl dyes	Contain one or more carbonyl groups linked by a quinonoid system	vat
Indigoid dyes	Contain indigoid chromogen	vat (only indigo and tetrabromo indigo are of commercial importance)
Polymethine and related dyes	Contain conjugated system of double bonds not in aromatic rings	basic
Styryl dyes	Contain styryl, C=C, group	disperse
Di- and tri-aryl carbonium dyes	Contain di- or tri-aryl substituted carbon atom	basic, related structures found in direct and reactive classes
Phthalocyanine dyes	Contain metal complex phthalocyanine chromogen	direct, reactive, acid
Quinophthalone dyes	Contain quinophthalone chromogen	disperse
Sulphur dyes	Contain sulphur atoms bridging aromatic ring structures	sulphur
Nitro and nitroso dyes	Contain nitro group on aromatic ring	acid, disperse
Miscellaneous dyes	Stilbene, formazan structures	direct, reactive

1.1.2 Application classification

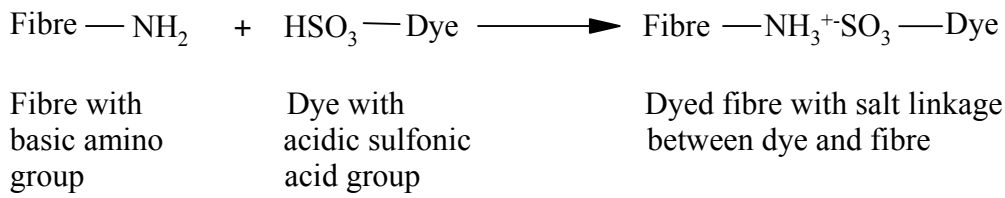
Classification by application method is most useful to the technologist concerned with colouration of textile products (Perkins, 1996). There are eight major classes according to method of application. Three dye classes used mainly for protein and synthetic fibres are acid dyes, basic dyes and disperse dyes. Five dye classes applied to dye on cellulose fibres are direct dyes, azoic dyes, sulphur dyes, reactive dyes and vat dyes. The characteristic of each of these classes is shortly brief in the following section.

1.1.2.1 Dyes for protein and synthetic fibres

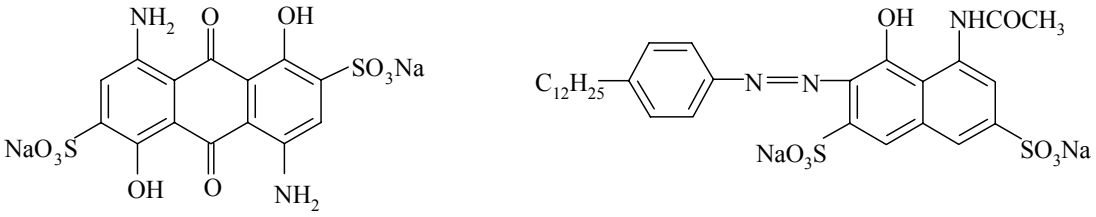
Protein fibres obtained from animal sources are mainly silk and wool. The structure of protein is a complex polypeptide containing a range of side-chains derived from the constituent amino acids (Christie *et al*., 2000). There are three important synthetic fibres, polyester, polyamides (nylon) and acrylic fibres, which have been dyed with disperse dyes and basic dyes. Three classes of dyes for protein and synthetic fibres will be discussed as follows:

Acid dyes contain acidic groups in their structure, usually sulphonate ($-SO_3^-$) groups, either as $-SO_3Na$ or $-SO_3H$ groups, although −COOH groups can sometimes be incorporated (Horrocks and Anand, 2000). The most common structural types of acid dyes are monoazo and anthraquinone. They have been used to dye protein fibres such as wool and silk. Wool, silk and other protein-based natural fibers have amino groups that can interact with acid dyes. The protein molecules carry a positive charge which attracts the acid dye anion by ionic forces as well as these ionic forces of attraction, van der Waal's forces, dipolar forces and hydrogen bonding between

appropriate functionality of the dye and fibre molecules may also play a part in the acid-dyeing of protein fibres (Perkins, 1996; Christie *et al*., 2000).



The most common structural types of acid dyes are anthraquinone and monazo as shown in Figure 1.2 (Perkins, 1996).

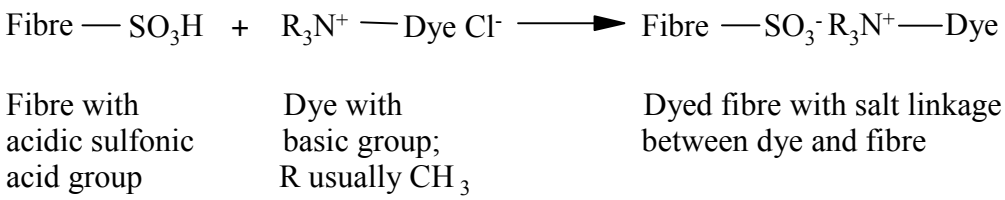


(a) (b)

Figure 1.2 Examples of acid dyes; (a) C.I. Acid Blue 45 and (b) C.I. Acid Red 138.

Basic dyes are cationic dyes containing a positive charge in their structure (Horrocks and Anand, 2000). Examples of basic dyes are given in Figure 1.3. They have been used to dye on fibres containing acidic groups that can interact with these cationic groups on their chromophore. The fibres contain either carboxyl (−COOH) or more commonly sulfonic acid ($-SO_3H$) groups (Perkins, 1996). After application to fibres, the interaction between the dye and fibres are salt linkages (Perkins, 1996).

The fastness properties of basic dyes are poor on wool and other protein fibres. On the other hand, the basic dyes show outstanding washfastness and lightfastness on acrylic fibres (Perkins, 1996).



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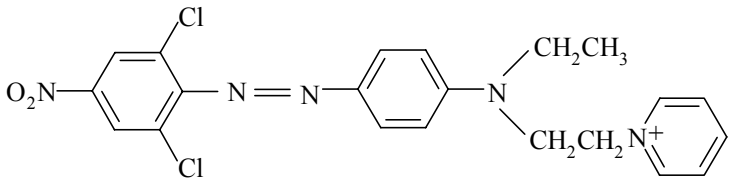

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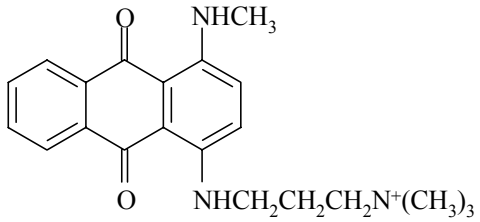


Figure 1.3 Examples of basic dyes; (a) C.I. Basic Orange 30:1 and (b) C.I. Basic Blue 22.

Disperse dyes are dyes of relatively low water-solubility applied as a fine dispersion in water (Christie *et al*., 2000). The major chemical classes used are aminoazobenzene, anthraquinone, nitrodiphenylamine, quinophthalone, styryl (methine), and benzodifuranone-based dyes (Horrocks and Anand, 2000). They are applied to relatively non-polar (hydrophobic) fibres, especially to polyester (Christie *et al*., 2000). An example of disperse dyes is illustrated in Figure 1.4.

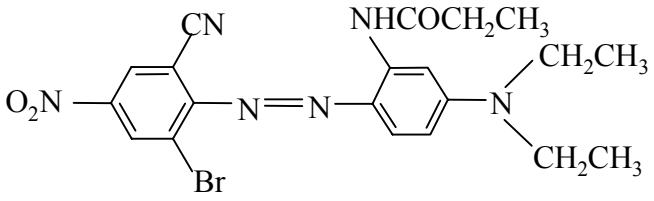


Figure 1.4 An example of disperse dye; C.I. Disperse Blue 183.

1.1.2.2 Dyes for cellulosic fibres

Cellulosic fibres such as cotton, viscose rayon and flax (linen) are natural fibres obtained from plant sources (Christie *et al*., 2000). They are composed of the polymeric carbohydrate, cellulose. Five of the most important dyes are used to dye cellulosic fibres as follows (Perkins, 1996):

Direct dyes are applied to dye on cellulose directly without using any auxiliary chemicals (Perkins, 1996). They are anionic dyes because of the presence of sulphonate ($-SO_3^-$) groups providing the water solubility (Christie *et al*., 2000). The most important feature of direct dye molecules which influence their application properties is their size and shape. In general, they are large molecules and long, narrow and planar in shape as illustrated in Figure 1.5. Direct dyes also generally contain groups such as amino, or hydroxyl groups which can interact to cellulosic fibres by forming hydrogen bonds (Perkins, 1996).

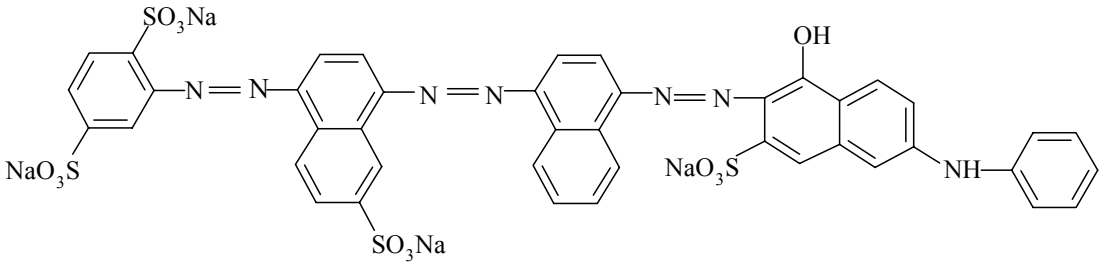


Figure 1.5 An example of direct dye; C.I. Direct Blue 78.

Azoic dyes account for over half of all commercial dye used in traditional textile applications. Azo colourants contain as their common structural feature the azo (−N=N−) linkage which is generally attached to at least one but most commonly two aromatic groups in trans configuration about the azo group (Christie *et al*., 2000). They provide a wide range of colours of high intensity and brightness. The azo dye structure contains one (monoazo), two (diazo), three (triazo) or more such groups (Christie *et al*., 2000). Azo dyes are purely synthetic and have no natural counterparts. An example of azo dye is shown in Figure 1.6.

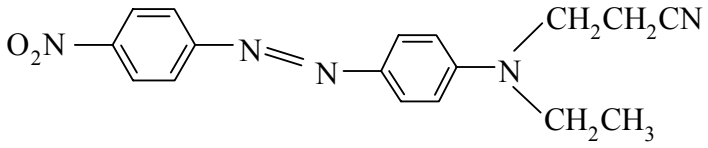
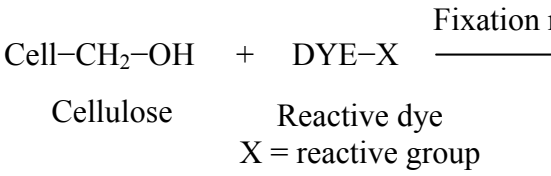


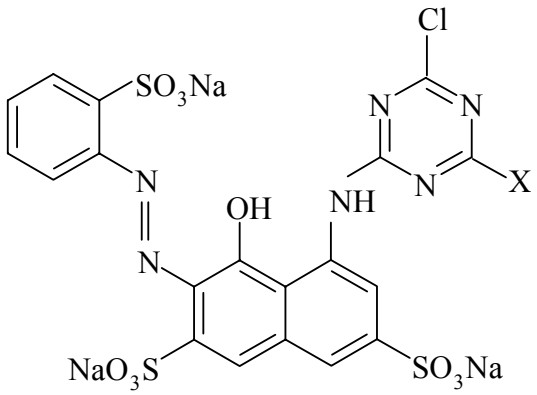
Figure 1.6 An example of a monoazo dye; C.I. Disperse Orange 25.

Sulphur dyes are complex mixtures of polymeric species containing sulphur in the form of sulphide (−S−), disulphide (−S−S−) and polysulphide (−S_n−) links and in

heterocyclic rings (Christie *et al*., 2000). However, the exact structure of the chromogen in sulphur dyes is difficult to establish. These dyes create the environmental problems because of sulphide residues in the effluent (Christie *et al*., 2000). Therefore, they are used less than other dye classes.

Reactive dyes consist of a chromophore attached to a reactive group through a −NH− group (Perkins, 1996) as shown in Figure 1.7. They are fixed to the cellulosic fibres by covalent bonds providing an excellent washfastness (Christie *et al*., 2000). The reaction between the dye and the fibres is a nucleophilic displacement (Perkins, 1996) as shown below:





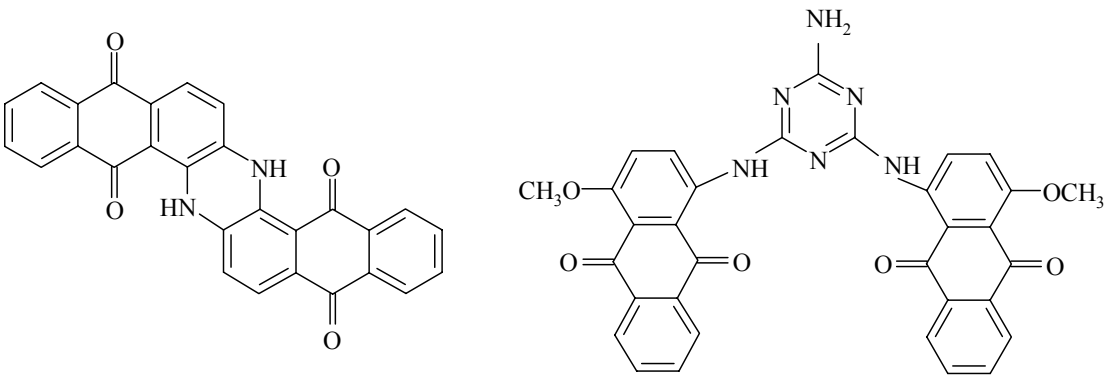
Chromophore ----- NH − Reactive group

Reactive dye structure schematic

(a) X = Cl; (b) X = NHPh

Figure 1.7 Examples of reactive dyes; (a) C.I. Reactive Red 1 and (b) C.I. Reactive Red 3.

Vat dyes are usually categorized into the following three groups: indigoid, anthraquinone and fused ring polycyclic (Perkins, 1996). Examples of vat dyes are shown in Figure 1.8. They are generally large planar molecules to provide the leuco form of the dyes with some affinity for the cellulosic fibres. The chemistry of vat dyeing is illustrated in Figure. 1.9 requires the presence in the vat dyes of two carbonyl groups linked via a conjugated system (Christie *et al*., 2000). The carbonyl groups must be reduced under alkaline conditions giving the leuco form water solubility (Christie *et al*., 2000). The step of dissolving the dye is called vatting. The dyes can be absorbed by the cellulosic fibres. Subsequently, they are converted back by an oxidation reaction to insoluble pigment. Therefore, the pigment produced becomes trapped within the fibre and its insolubility gives rise to the excellent washfastness properties which are characteristic of vat dyes.



(a) (b)

Figure 1.8 Examples of vat dyes; (a) C.I. Vat Blue 4 and (b) C.I. Vat Red 28.

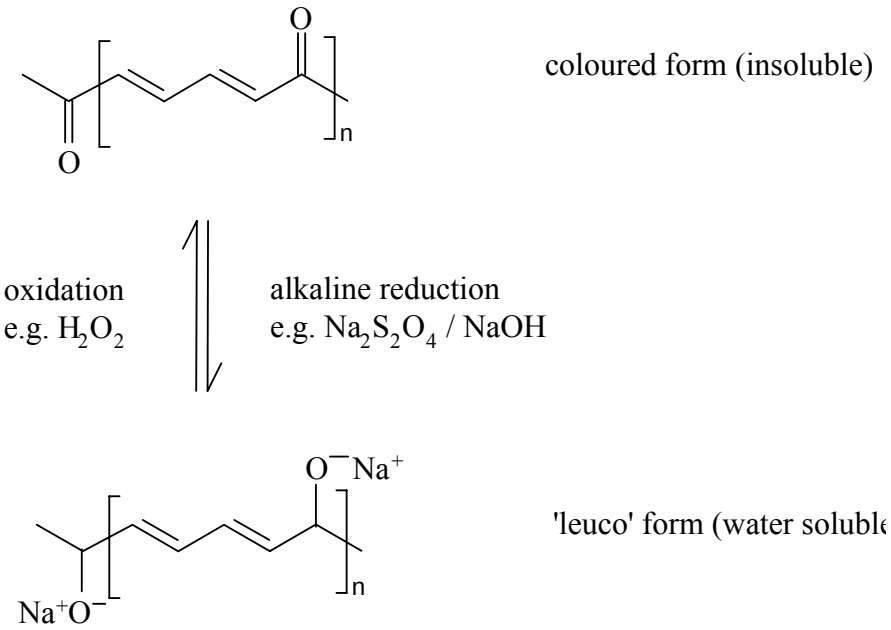


Figure 1.9 The chemistry of vat dyeing.

Nowadays, the synthetic dye in dyeing process results in an environmental pollution (Rastogi, Gulrajani and Gupta, 2000). Many synthetic dyes may lead to various harmful byproducts during their dyeing manufacture. A number of azo dyes which release carcinogenic harmful amine have already been banned by most countries. Furthermore, the effluent discharged in a dyeing process is also causing a lot of concern. Therefore, there is an increasing realization in the textile industry as well as among the textile consumers of the need to develop and demand eco-friendly methods of dyeing textile. Natural dyes offer an important alternative in this regard, as these are safer in use with minimum health hazards, have easy disposal and cause less disposal problems. Nowadays, there is interest in dyeing of textile fibres with natural dyes, on account of their compatibility with the environment, and because of

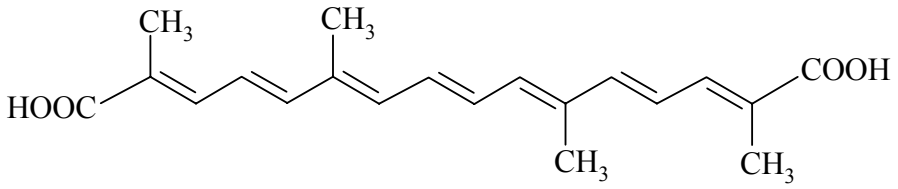
their lower toxicity and allergic reactions. The natural dye will be discussed as follows.

1.2 Natural dyes

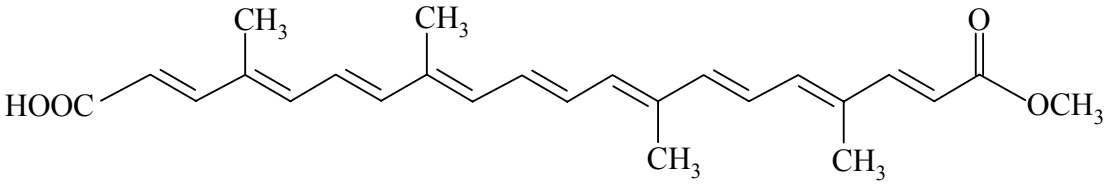
The natural dyestuffs used to dye clothing were commonly extracted either from vegetable sources, including plants, trees, roots, seeds, nuts, fruit skins, berries and lichens, or from animal sources such as crushed insects and mollusks (Christie, 2001). The important natural dye classes from plant sources are chlorophyll, carotenoids, flavonenoids, and quinones (Lemmens and Wulijarni-Soetjipto, 1992) as given below:

Chlorophyll is a green compound found in leaves and green stems of plants (Lemmens and Wulijarni-Soetjipto, 1992).

Carotenoids are the most widely occurring in the animal and vegetable kingdoms (Freeman and Peters, 2000). They are mostly fat-soluble, nitrogen-free yellow to reddish violet dyes, which contain a long chain of conjugated double bonds, and so constitute aliphatic polyenes. Examples of carotenoids dye are C.I. Natural Yellow 6 and C.I. Natural Orange 4 as shown in Figure 1.10.



(a)



(b)

Figure 1.10 Example of carotenoids; (a) C.I. Natural Yellow 6 and (b) C.I. Natural Orange 4.

Flavonoids constitute most of the yellow, red and blue colors in flowers and fruits. The flavonoids include the following subgroups namely chalcones, flavanones, flavones, flavonols, anthocyanins and isoflavonoids (Lemmens and Wulijarni-Soetjipto, 1992). Examples of flavonoid pigments are morin and rutin as shown in Figure 1.11.

Quinones are characterized chemically by the presence a quinone group (Figure 1.12). They are capable of providing a yellow to red shade. The important subgroups are benzoquinones, naphthoquinones and anthraquinones (Lemmens and Wulijarni-Soetjipto, 1992). Examples of anthraquinones are alizarin, morindin and purpurin found in family *Rubiaceae* (Figure 1.12).

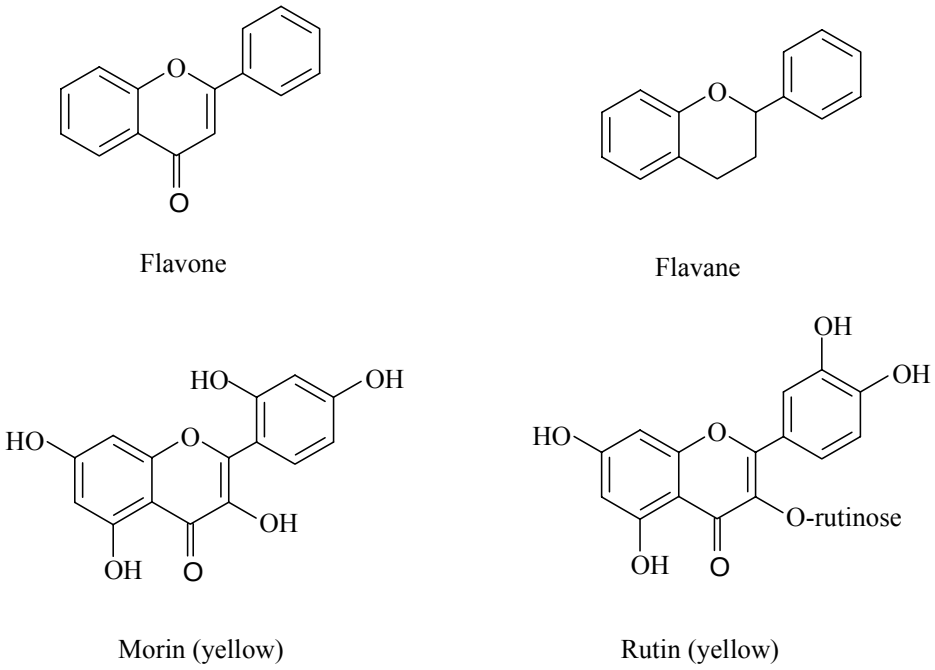


Figure 1.11 Basic structures (flavone and flavane) of most flavonoids, and examples of flavonoid pigments: morin and rutin.

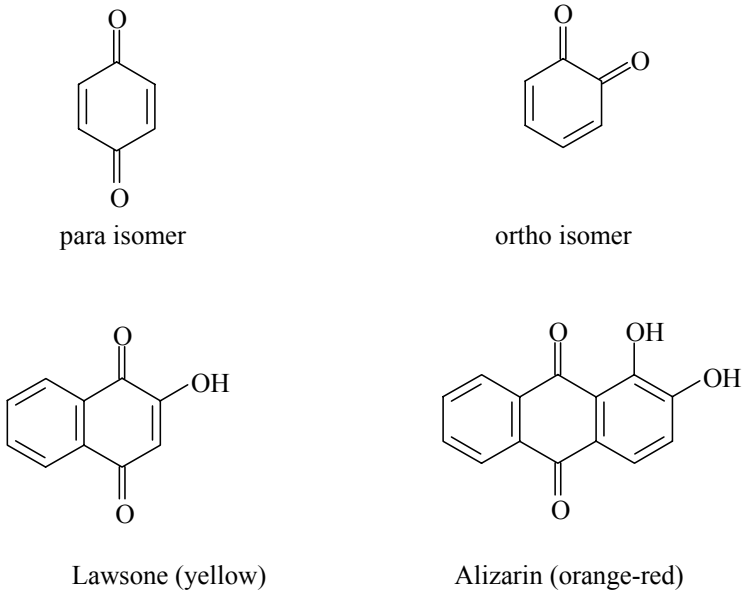


Figure 1.12 Structure of quinone and examples of a naphthoquinone (lawsone) and an anthraquinone dye (alizarin).

Lac dye is a natural reddish dyestuff extracted from stick lac, which is a secretion of the insect *Coccus laccae* (*Laccifer lacca* Kerr) (Freeman and Peters, 2000). It is categorized into the quinone group. In general, the insect *Coccus laccae*, together with the other lac insect species (*Lakshadia (Tachardia, Laccifer)* spp.), is found in South and Southeast Asia, especially in Thailand and India (Schweppe, 1989). In Thailand, lac insect grows most commonly on the Rain tree, *Samanea saman* (Jacq.) Merr. (*Pithecolobium saman,* Mimosaceae) (Moeyes, 1993). However, the lac insect also grows on *Combretum quadrangulare* Kurz (Combretaceae) and on *Dalbergia cochinchinensis* Pierre (Fabaceae) but only rarely. The lac insect looks like a mite as shown in Figure 1.13. The female insect has a short and rounded body whereas the male insect has a long body. They have six legs. Lac insects settle closely on the twigs of certain host trees, suck the plant sap and grow, all the while secreting lac resin from their bodies. Since the insects are closely spaced on the twigs, the resin forms continuous encrustations over the twigs of the host trees. The resin is called the stick lac. It is only the female insects that produce the red dyestuff. The red colour is derived from a water-soluble part of stick lac which consists of laccaic acids A, B, C, D, E and F (Figure 1.14). All of these acids have an anthraquinone moiety with dicarboxylic acid groups, except for laccaic acid D which is a monocarboxylic acid (Yamada, Noda, Mikami, Hayakawa and Yamada, 1989; Budavari, 1996). The quantities of these components depend on the locality and the season. The chemical structures of the laccaic acids are shown in Figure 1.14. Lac dye has been used to dye on the silk and cotton in our study.

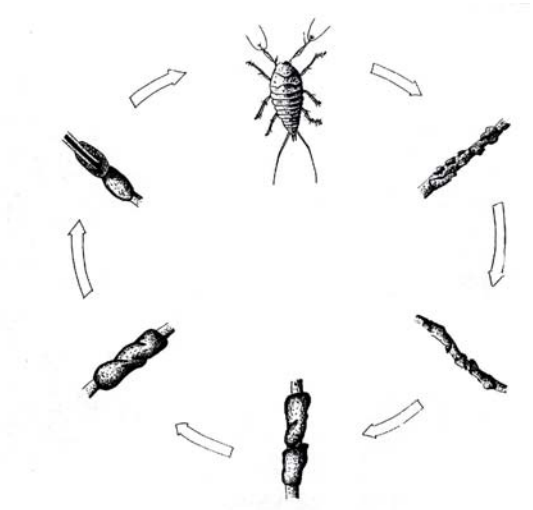
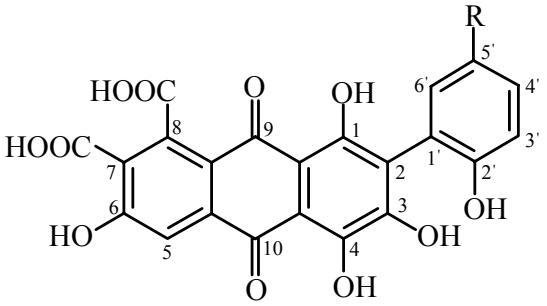
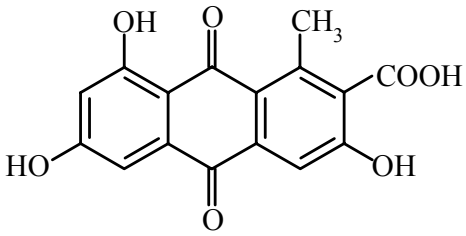


Figure 1.13 The life cycle of the insect *Coccus laccae* (*Laccifer lacca* Kerr) (Moeyes, 1993).





Laccaic acid A	R = $CH_2CH_2NHCOCH_3$	MW 537	Laccaic acid D	MW 314
Laccaic acid B	R = CH_2CH_2OH	MW 496		
Laccaic acid C	R = $CH_2CHCOOH$ (NH$_2$ on CH)	MW 539		
Laccaic acid E	R = $CH_2CH_2NH_2$	MW 495		
Laccaic acid F	R = $CH_2CH_2OCOCH_3$	MW 538		

Figure 1.14 Chemical structures of the laccaic acids.

1.3 Fibres

All fibre-forming molecules consist of a long chain of molecules (Horrocks and Anand, 2000). The classification of textile fibres is based on the principle origin of the fibre (natural or man-made), chemical type (cellulosic, man-made cellulosic), generic term (seed, hair, rayon) and common names and trade names of the fibres (cotton, viscose, rayon) (Karmakar, 1999). For the application of dyes, a simpler classification into three broad categories often suffices (Christie *et al*., 2000):

- *Protein fibres* e.g. wool and silk;
- *Cellulosic fibres* e.g. cotton, viscose rayon and flax (linen);
- *Synthetic and cellulose acetate fibres* e.g. polyester, polyamide, acrylic and cellulose triacetate.

Some important fibres that mentioned above are shortly brief in the section 1.3.1 and 1.3.2.

1.3.1 Protein fibres

Protein fibres derived from animal sources are wool and silk. The chemical structure of silk is only discussed in this section. Silk fibre is a fine continuous strand unwound produced naturally by a moth caterpillar known as silkworm, *Bombyx mori* (Karmakar, 1999; Carr, 1995). The silkworm spins cocoon around its body by extruding the contents of the two silk glands through a spinnerette at its mouth. The two filaments solidify on coming in contact with air and form a composite thread. Silk has high tenacity, high luster and good dimensional stability. There are mainly four varieties of silk, e.g. Mulbery, Tassar, Eri and Muga (Karmakar, 1999). All the species of silk have four stages in their life cycles namely, the eggs, larva, pupa and moth. The mulberry silkworm belongs to the species *Bombyx mori*

and about 95% of the world's production is of this species. Raw silk thread is obtained from silk cocoons by reeling after boiling the cocoons in water.

The actual fibre protein is called fibroin and the protein sericin is the gummy substance that holds the filament together. Raw silk has an average composition of 70-75% fibroin ($C_{15}H_{23}N_5O_8$), 20-25% sericin, 2-3% waxy and 1 to 1.7% mineral matter (Karmakar, 1999). Sericin is amorphous and removed by dissolving in hot soap solution. Fibroin is the form of a filament tread and dissolves in 5% sodium hydroxide solution at boil.

Both fibroin and sericin are protein substances built up of 16-18 amino acids out of which only glycine, alanine, serine and tyrosine make up the largest part of the silk fibre and the remaining amino acids containing bulky side groups are not significant (Karmakar, 1999). The chemical structure of fibroin and sericin for four amino acids is shown in Table 1.2.

Structures of *Bombyx mori* silk fibroin have been studied by using solid ^{13}C- and ^{15}N-NMR spectroscopies (Kaplan, Adams, Farmer and Viney, 1994). It indicated that main sequence of silk fibroin is Gly−Ala−Gly−Ala−Gly−Ser where Gly, Ala and Ser denote glycine, alanine and serine respectively (Kaplan *et al*., 1994). In addition, the crystal of silk fibroin was reexamined by using newly collected intensity data. It was found that the crystalline region of silk is composed of rather irregular stacking of the antipolar-antiparallel beta sheets with different orientation. For the dyeing process, silk fibres contain amino groups ($-NH_2$) which in an acid dye bath are protonated to give $-NH_3^+$ groups (Horrocks and Anand, 2000). A synthetic ionized such as acid dye is thus attracted to the silk and is adsorbed, forming an ionic linkage, with the fibre' s

dye sites. Lac dye used in our research can probably bind to the protonated dye sites ($-NH_3^+$) of the silk fibres in the same way.

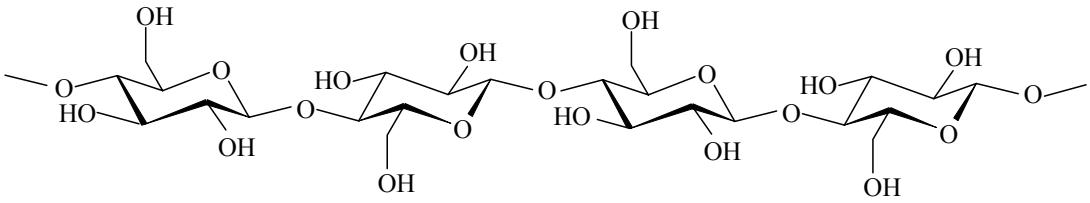
Table 1.2 Amino acid composition of sericin and fibroin (Karmakar, 1999)

Amino acids	Side groups	Sericin (% mol)	Fibroin (% mol)
Glycine	H −	14.75	45.21
Alanine	CH_3 −	4.72	29.16
Serine	$CH_2(OH)$ −	34.71	11.26
Tyrosine	$OHC_6H_4CH_2$ −	3.35	5.14

1.3.2 Cellulosic fibres

Cellulose fibres are natural fibres derived from plant sources. The most important cellulosic fibres are cotton, viscose, linen, jute, hemp and flax (Christie, 2001). The principal component of these fibres is cellulose, a high molar mass, linear polymeric sugar or polysaccharide. The polymer is formed from molecules of the monosaccharide (Christie *et al*., 2000), β-D-glucose linked through carbon atoms in the 1- and 4-positions. Cotton accounts for half of the world's consumption of fibres (Horrocks and Anand, 2000). It is the major textile fibre which consists of practically pure cellulose about 88-96% of the fibre (Lewis; 1993). Cotton may be chemically described as poly (1,4-β-D-anhydroglucopyranose) (Karmakar, 1999) (Figure 1.15). Large dye molecules can be penetrated easily into the fibre because cellulose has a fairly open structure (Christie, 2001). There are three hydroxy groups in each glucose

unit of the cellulose structure, two of which are secondary and one primary, and these give the cellulose molecule a considerable degree of poly character (Christie, 2001).



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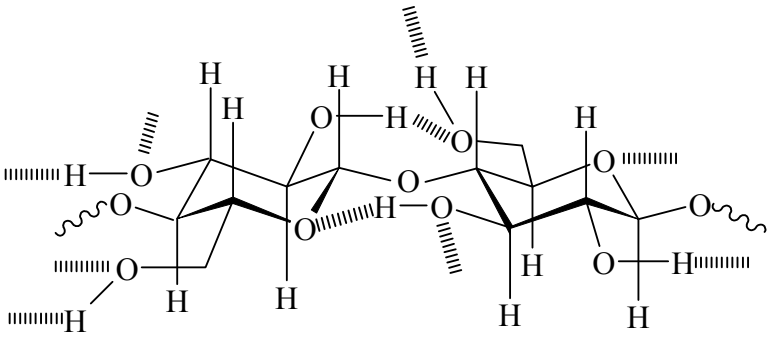


(b)

Figure 1.15 The structure of cellulose; (a) cellulose structure and (b) hydrogen bonding in cellulose structure.

The presence of the hydroxy groups is of considerable importance in the dyeing of cotton. For example, the ability of the hydroxy groups to form intermolecular hydrogen bonds is thought to be of some importance in direct dyeing, while reactive dyeing involves a chemical reaction of the hydroxy groups with the dye to form dye- fibre covalent bonds (Christie, 2001). The tendency of the hydroxy groups to ionize to a certain extent (to $-O^-$) means that the fibres can carry a small

negative charge (Christie, 2001). There are a larger number of application classes of dyes that may be used to dye cellulosic fibres such as cotton than for any other fibre. Direct, azoic, sulphur, reactive and vat dyes are coloured on cellulosic fibres as discussed in the section 1.1.2.2.

The red colour from lac dye is used extensively for natural food additives (Ikawa and Yoshizeki, 1981; Yamada *et al*., 1989; Zollinger, 1991; Ishigami and Suzuki, 1997; Watanabe and Terabe, 2000), cosmetics and as a colourant for silk and cotton dyeing (Moeyes, 1993). It is believed that the Chinese used lac to dye silk cloth for more than 4,000 years ago (Moeyes, 1993), well before any other countries. "Yi Chi Chik Hsu" is the name given to an ancient piece of silk cloth that was dyed red with lac. In Europe it was already in use in 3,700 B.C. The ancient Greeks used lac to colour pieces of pottery. The Assyrians knew how to dye cloth with lac, while the Gauls also used lac to dye their woven cloth.

In Thailand, it is used as a natural red dyestuff for cotton and silk dyeing (Conway, 1992; Moeyes, 1993) but the fastness properties and reproducibility to give consistency in production are still problems to be solved. Improvements and research on the chemistry of dyeing and the adsorption mechanism may help solve these problems. The successful improvement of the dyeing process will improve the economic well-being of villagers in the north-eastern part of Thailand who are mostly poor.

The aims of this study were to extract, purify, characterize, and quantify the components of lac dye from stick lac in Thailand using suitable methods because lac components extracted from Thai stick lac had not been reported. The kinetics and thermodynamics of lac dyeing on cotton and silk were to be investigated in order to

obtain the parameters of lac dyeing on cotton and silk. In addition, the interactions of lac dye with metal ion mordants were also to be studied. In order to gain a greater understanding of the action of these mordants, a systematic study of the interaction of lac dyes and laccaic acids with some metal ions was undertaken.

1.4 Research objectives

(a) To extract, purify, characterize, and quantify the components of Thai lac dye from lac insects on the Rain tree.

(b) To study the kinetics and thermodynamics of lac dyeing on cotton and silk.

(c) To study the interactions of lac dye with metal ion mordants and/or non-metal mordants.

1.5 Scope and limitations of the study

Lac material will be collected from the Rain tree and standard laccaic acids A, B and C will be purchased from Japan (Wako Company). Identification and structural elucidation of the extracted lac dye will be done using different techniques such as nuclear magnetic resonance spectroscopy (NMR), Fourier transform infrared spectroscopy (FI-IR) and mass spectrometry (MS). Kinetic and thermodynamic studies of lac dyeing on cotton and silk will be performed using techniques such as UV-Visible spectroscopy.

CHAPTER II

ANALYSIS OF LACCAIC ACIDS FROM THAI STICK LAC

2.1 Introduction

Over recent decades, several researchers using different methods, have isolated and characterized the components of lac dye extracted from stick lac. In India, Pandhare, Rama Rao, Srinivasan and Venkataraman (1966) extracted lac dye with water and passed it through a column of cation-exchange resin (H form) from cashew-nut shell liquid. The extracted lac dye was separated into two main fractions as pure homogeneous compounds on a polyamide column using 1-butanol saturated with 0.3 M HCl as eluant. Laccaic acid A, which was the major fraction, was methylated with dimethyl sulfate and potassium carbonate in boiling acetone (Pandhard *et al.*, 1966). The ^{1}H-NMR studies showed that laccaic acid A was 1,3,4,6-tetrahydroxy-2-[2′-hydroxy-5′-(β-acetamidoethyl)-phenyl]-9,10-anthraquinone-7,8-dicarboxylic acid as shown in Figure 2.1 (Pandhare, Rama Rao and Shaikh, 1969). Therefore, the molecular formula of laccaic acid A was determined to be $C_{26}H_{19}NO_{12}$ and the acid appeared to be identical with Burwood's laccaic acid A_1 (Burwood, Read, Schofield and Wright, 1967). The UV-VIS absorption spectrum of laccaic acid A had a close resemblance to that of crude laccaic acid in ethanol, thereby showing that the main chromophore of its constituents was likely to be the same. Laccaic acid A in ethanol

showed peaks at λ_{max} 290 (log ε 4.8), 340 (log ε 4.31), 500 (log ε 4.32) and 530 (log ε 4.12) nm in the UV-VIS absorption spectrum (Pandhare *et al*., 1969). The infrared spectrum of laccaic acid A in nujol showed absorption peaks at 1715, 1692, 1667 and 1626 cm^{-1}. The 1692 and 1715 cm^{-1} peaks can be assigned to carboxylic acid groups, the peak at 1626 cm^{-1} to chelated quinone carbonyl groups as in quinizarin and purpurin, and the 1667 cm^{-1} peak to an additional carbonyl group in an amide. In addition, analysis of the curve representing the neutralization of laccaic acid A_1, in water containing 4% of dimethyl sulphoxide, by 0.1 M sodium hydroxide indicated pKa_2 and pKa_3 to be about 4.4 and 6.5 respectively (Burwood *et al*., 1967). The most strongly acidic group was completely ionized below pH 4.0. Therefore, the pKa_1 of laccaic acid A_1 must be very low. The weakest acidic group in this compound was the 3-hydroxy group whereas the two stronger acidic groups were carboxylic acid groups.

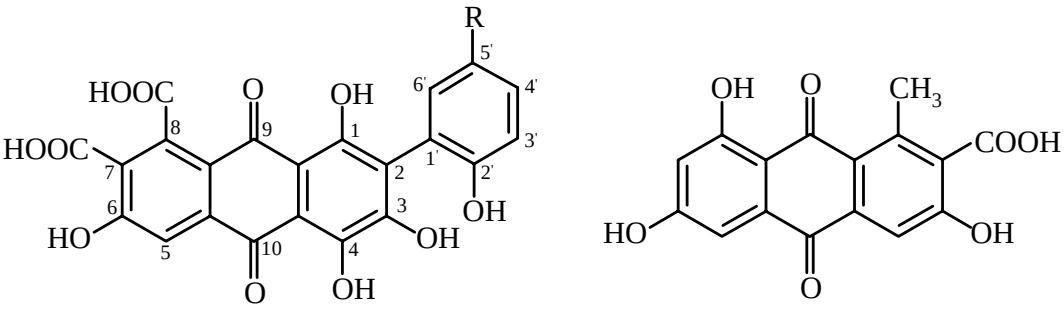
The minor fraction which was isolated on the polyamide column using 1-butanol saturated with 0.3 M HCl was laccaic acid B (Pandhare, Rama Rao, Shaikh and Venkataraman, 1967; Bhide, Pandhare, Rama Rao, Shaikh and Srinivasan, 1969). The constitution of laccaic acid B was based on physical and chemical methods, and was shown to be 1,3,4,6-tetrahydroxy-2-[2′-hydroxy-5′-(β-hydroxyethyl)-phenyl]-9,10-anthraquinone-7,8-dicarboxylic acid as shown in Figure 2.1, the non-nitrogenous constituent of laccaic acid from stick lac. The electronic spectrum of laccaic acid B was similar to laccaic acid A in the UV and VIS regions, indicating that the two acids had the same chromophore. The infrared spectrum of laccaic acid B in nujol showed absorption bands at 1695 and 1712 cm^{-1} that were assigned to carboxyl groups. In addition, the peak at 1618 cm^{-1} confirmed the presence of the chelated quinone carbonyl groups. (Bhide *et al*., 1969).

Laccaic acid C has been isolated from stick lac according to the method of Rama Rao, Shaikh and Venkataraman (1968). In their method, the laccaic acids were separated on a column of cellulose powder using 1-butanol–acetic acid–water (6:1:2). The major components, laccaic acids A and B were observed as a single band and minor fractions were also collected. The minor fractions were separated into two bands that were laccaic acids C and E, respectively. They reported that the laccaic acids C and E that were separated from stick lac constituted about 5% of the total dye. Their UV-VIS spectra and colour reactions closely resembled laccaic acids A and B but they exhibited a clear ninhydrin reaction. The data from the ^{1}H-NMR spectrum of methylated laccaic acid C showed that the structure of laccaic acid C was similar to laccaic acids A and B. However, it differed from laccaic acids A and B in the side chain, which was an α-amino acid instead of CH_2CH_2NHAc (laccaic acid A) or CH_2CH_2OH (laccaic acid B) (Figure 2.1).

Laccaic acid D from Rangini stick lac (host trees *Butea monosperma* and *Zizyphus mauritiana*) were separated by using silica gel column chromatography with acetone as eluant (Mehandale, Rama Rao, Shaikh and Venkataraman, 1968). A fast moving yellow band obtained by column chromatography was laccaic acid D. Analysis of the ^{1}H-NMR spectrum of an ether-ester derivative obtained by methylation with diazomethane in dry ether of laccaic acid D indicated the presence of three aromatic protons, four methoxy groups and a C-methyl group. It was found that laccaic acid D was identical in all its properties with xanthokermesic acid. The chemical structure of laccaic acid D is shown in Figure 2.1

Laccaic acid F, new red pigment, isolated from Thai stick lac was reported by Hu, Hasegawa and Nakatsuka (1997). It was isolated by a combination of silica gel

column chromatography (1% $(CO_2H)_2$ / EtOAc), preparative TLC (1% $(CO_2H)_2$ / EtOAc) and HPLC (Namsil ODS-9, 50% MeOH / H_2O). The chemical structure is shown in Figure 2.1.



Laccaic acid A R = $CH_2CH_2NHCOCH_3$ MW 537 Laccaic acid D MW 314

Laccaic acid B R = CH_2CH_2OH MW 496

Laccaic acid C R = $CH_2CHCOOH$ (NH$_2$) MW 539

Laccaic acid E R = $CH_2CH_2NH_2$ MW 495

Laccaic acid F R = $CH_2CH_2OCOCH_3$ MW 538

Figure 2.1 Chemical structures of the laccaic acids.

In Bangladesh, Haque, Faraq and Ali (1998) isolated lac dye from stick lac of Bangladeshi origin. Lac dye was extracted with deionized water at room temperature and then precipitated as the calcium salt. The mixture of extracted lac dye, obtained after acidification of the salt, was purified on a silica gel column. TLC analysis showed the presence of a new laccaic acid, A_1, as well as laccaic acids B and C (R_f 0.75, laccaic acid A_1; R_f 0.85, laccaic acids B+C; silica gel; 1-butanol–acetic acid–water, 4:1:5). They proposed the molecular structure of laccaic acid A_1,

$C_{20}H_{14}O_{10}$ (Figure 2.2), based on the physico-chemical properties and spectral analysis.

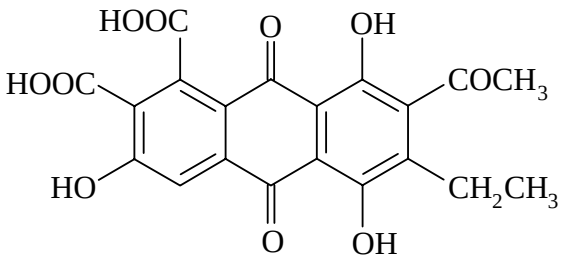


Figure 2.2 Chemical structure of laccaic acid A_1.

In Japan, Oka *et al*. (1998) were able to separate the lac dye components by high-speed counter-current chromatography using a two-phase solvent system composed of *tert*-butyl methyl ether–1-butanol–acetonitrile–water (2:2:1:5). Each component in the fractions was identified by high-performance liquid chromatography and electrospray tandem mass spectrometry. It was found that the separation from 25 mg of lac sample yielded 2.6 mg of 97.2% pure laccaic acid C, 9.5 mg of 98.1% pure laccaic acid A, 3.6 mg of 98.2% pure laccaic acid B and 0.5 mg of a 95.0% pure anthraquinonedicarboxylic acid with a molecular mass of 360. The structure of this last acid was not determined.

Apart from the isolation of lac dye components, various methods have been developed for the detection of lac colour in food. For example, Oka *et al*. (1997) have successfully identified laccaic acids in jelly by an electrospray high performance liquid chromatography-mass spectrometry (ESI LC/MS/MS) method. Yamada, *et al*. (1989) detected lac dye in food using methylation of lac dye with diazomethane. In

their experiment, lac colour was extracted with methanolic oxalic acid and eluted from a column of Amberlite XAD-2 with the same solvent. The fraction containing the lac dye was treated with diazomethane to produce two reddish-orange markers. The marker species in the reaction mixture were detected by both thin-layer chromatography and reverse-phase liquid chromatography. It was found that the reddish-orange markers were completely distinguished from other spots on a silica gel plate with a chloroform–methanol–water (90:10:1, v/v) as the developing solvent. Hisada, Terada and Miyabe (1999) extracted lac pigments in food using ethanol–water–oxalic acid and then purified them on a polyamide immobilized column. They then investigated the lac dye components by thin layer chromatography and high-performance liquid chromatography. Nishizawa, Chonan and Hori (1985) isolated laccaic acids A [15979-35-8] and B [17249-00-2] in commercial lac dye and food by liquid chromatography using a Nucleosil 5 C18 column with MeCN-5%H_3PO_4 as the mobile phase. It was found that commercial lac dye contained 1.66-1.69% laccaic acid A and 0.40-0.42% laccaic acid B while food crackers contained 3.7 μg/g laccaic acid A.

In addition, Kozu (1979) has successfully increased the quality of laccaic acid [60687-93-6]. Stick lac was immersed in methanol at room temperature, the methanol extract dried under reduced pressure, and the residue then extracted with water at pH 4.0-4.5. The aqueous solution was then concentrated under reduced pressure, dried and immersed in 80-85 vol. % ethanol/water at less than 60 ^{o}C. The solution in ethanol was filtered, evaporated and the residue extracted with water pH 4.0-5.5. The pH of the aqueous extract was then lowered to precipitate laccaic acid. Laccaic acid obtained by this procedure showed high quality in purity and colour. The UV-VIS

spectrum of laccaic acid A is very similar to the spectrum of crude laccaic acid in ethanol (Burwood *et al.*, 1967) with absorption maxima for laccaic acid A reported at 290, 340, 500 and 530 nm in this solvent (Burwood *et al*., 1967); in water, λ_{max} values were reported at 292 and 490 nm and at 500 nm in acetone for this acid (referred to as laccaic acid (I)) (N. Prasad, K. M. Prasad, Ghosh and Khanna, 1984).

Aims

The aims of this part of the project were to extract, purify, characterize, and quantify the components of lac dye from Thai stick lac collected in Northeast Thailand using suitable methods.

In recent years, there have been numerous studies on the use of ultrasonication for assisting the extraction of organic compounds from various matrices. For instance, the ultrasonically assisted extraction of the water-soluble polysaccharides from the roots of valerian (*Valeriana officinalis L.*) has been reported by Hromadkova, Ebringerova and Valachovic (2002). Also, the ultrasonication method has been applied to the extraction of bioactive principles from herbs (Vinatoru, 2001), the bio-available fraction of humic substance in marine sediments (Mecozzi, Amici, Pietrantonio and Romanelli, 2002) and chlorogenic acid from *Eucommia ulmodies* Oliv. (*E. ulmodies*) (Li, Chen and Yao, 2004). It is believed that ultrasonication accelerates the extraction of organic compounds from plant materials due to disruption of cell walls and enhanced mass transfer of the cell contents (Hromadkova *et al*., 2002). Apart from ultrasonication, microwave-assisted extraction has also been shown to enhance the extraction efficiency of components from a wide variety of

sample matrices. Microwave-assisted extraction has been used for example in the extraction of glycyrrhizic acid from the roots of *Glycyrrhixia glaubra* (Pan, Liu, Jia and Shu, 2000), the extraction of tanshinones form the dried roots of *Salvia miltiorrhiza bunge* (Pan, Niu and Liu, 2001) and the extraction of ginsenosides from the roots of *Panax ginseng* (Shu, Ko and Chang, 2003).

In general, lac dye has been extracted with water at room temperature (Burwood, Read, Schofield and Wright, 1965; Pandhare *et al*., 1966; Pandhare *et al*., 1969; Haque *et al*., 1998) and with hot water (50 ^{o}C) (Deb Roy and Pathak, 1971), and it was found that the laccaic acids represented about 1% by weight of stick lac (Burwood *et al*., 1965). In order to increase the extraction yield of lac dye, ultrasonication and the microwave-assisted methods were investigated in the present research to extract lac dye from Thai stick lac. A comparison with hot water (60-75 ^{o}C) extraction was also made. The ultrasonication and microwave-assisted methods for the extraction of lac dye have not been reported previously.

2.2 Experimental

2.2.1 Chemicals

All reagents and solvents were purchased from Sigma-Aldrich Chemical Co. Inc. and were used as received. Standard laccaic acids, Laccaic acid A [15979-35-8], laccaic acid B [17249-00-2] and laccaic acid C [23241-56-7], were purchased from the Wako Company (Osaka, Japan). Solvents were removed under reduced pressure at 40 ^{o}C with a Büchi rotary evaporator (Büchi Rotavaor R-114). All solvent mixture ratios are by volume.

2.2.2 Source of stick lac

Stick lac was purchased from Oun Kankaset shop, Pukthongchai District, Nakhon Ratchasima Province and from Nong-Ying shop, Muang District, Surin Province. It was grown on the Rain tree (*Samanea saman* (Jacq.) Merr. (*Pithecolobium saman*, Mimosaceae) and collected in November 2002 by villagers in northeast Thailand. Authentic samples of the stick lac used are deposited in the School of Chemistry, Suranaree University of Technology, Nakhon Ratchasima Province.

2.2.3 Extraction of lac dye

Lac dye derived from Thai stick lac was extracted using three methods as follows:

2.2.3.1 Ultrasonication-assisted extraction at 60 ^{o}C

The powdered stick lac (500 g) was extracted with deionized water (1.5 L) using an ultrasonic bath (Ultrasonic Cleaner, Model-575 HT, frequency 38.5-

40.0 kHz, the average power 135 Wattage, peak power 405, Crest Ultrasonics Corp., Trenton, NJ, USA) at 60 ^{o}C for 1 hour. The aqueous solution was filtered and then concentrated under reduced pressure (rotary evaporator) and finally dried using a freeze dryer to give a crude lac extract.

2.2.3.2 Microwave-assisted extraction at 60-70 ^{o}C

The powdered stick lac (100 g) was extracted with deionized water (300 mL) using a household microwave oven (Whirlpool, V100 model, 2450±50 MHz, full power 800 W, China). It was irradiated with microwave irradiation (400 W) for 3 minutes and then allowed to cool for 10 minutes with the power off (temperature about 60-70 ^{o}C). This irradiation/cooling cycle was repeated for a total of 5 cycles. The aqueous solution was filtered and then the filtrate concentrated under reduced pressure (rotary evaporator) and the residue dried using a freeze dryer to give a crude lac extract.

2.2.3.3 Hot water extraction at 60-75 ^{o}C

Stick lac was powdered in a grinding mill and finely ground (18 mesh). The powdered material (500 g) was extracted with deionized water (1.5 L) at 60-75 ^{o}C for 1 hour. The aqueous solution was filtered and then concentrated under reduced pressure (rotary evaporator) and the last traces of water then removed by freeze drying (Heto FD3 model S/N 492497-B, Cat No. 837107, Denmark) to give a crude lac extract.

2.2.4 De-fatting of crude lac extract

Crude lac extract (10 g) was de-fatted with boiling chloroform (300 mL) at reflux (60 ^{o}C) for 24 hours. After cooling, the solvent was filtered and then the de-fatted lac dye was dried overnight under high vacuum.

2.2.5 Separation of lac dye components

De-fatted lac dye (10 g) from each crude extract was extracted with methanol (300 mL) in a Soxhlet apparatus until the methanol in the Soxhlet was colourless (7 days). The methanol was evaporated at reduced pressure and then the residue was dried under high vacuum to constant weight. The lac dye obtained after extraction with methanol was stirred with methanol and cellulose powder. The solvent was evaporated under reduced pressure at 40 ^{o}C and then dried *in vacuo* for 12 hours. It was then slurried in a small volume of the organic phase from ethyl acetate, acetic acid and water (E:A:W, 4:1:5) and then introduced into a packed cellulose column (Cellulose microcrystalline powder, 20 micron, CAS # 9004-34-6, Aldrich Chemical Company, Australia). The column was eluted with ethyl acetate, acetic acid and water (E:A:W, 4:1:5) until the eluate was colourless (Burwood *et al*., 1965). The eluant was then changed to methanol, acetic acid and water (M:A:W, 90:5:5). The eluted fractions were dried by evaporation of the eluted solvents and then washed with water until the rinsed water was neutral. The residues were dried *in vacuo* to constant weight. The amount of dye that was used in these experiments is listed in Table 2.1.

Table 2.1 Weight of dye from each crude extract separated by chromatography on cellulose

Source	**Nakhon Ratchasima**	**Surin**	**Surin**	**Surin**
Extraction method	Hot water	Hot water	Ultrasonication	Microwave
Wt. of cellulose powder for packing column (g)	60	60	160	160
Wt. of lac dye (g) loaded on the cellulose column	1.09	1.02	2.63	3.59

2.2.6 Purification of laccaic acids

Water was added to the lac dye extract and then the aqueous dye solution was acidified with concentrated hydrochloric acid and kept for 24 hours. The precipitated laccaic acid was collected and then washed with water until the washings were neutral. The laccaic acid was then dried *in vacuo* until constant weight was achieved.

2.2.7 Chemical structural analysis of the purified laccaic acids

The purified laccaic acids were identified by HPLC analysis, mass spectrometric analysis, and NMR spectroscopy.

2.2.7.1 HPLC analysis

A Waters^{TM} 600 Controller (Waters, Australia) was used with a Waters 486 Tunable Absorbance Detector operated at 400 nm. The separation was performed

on an Econosil C18 (10 μm, 250 x 4.6 mm i.d. Part No. 40147, Lot No. 260882, Waters, Australia) reverse phase column with 50 mM $NH_4H_2PO_4$ – CH_3OH (85:15), pH 7.8 (adjusting the pH with 28% aqueous NH_3 solution) as the mobile phase at a flow rate of 0.80 mL/min. Two other solvent systems were also used as mobile phases:

(1) acetonitrile – 0.01 M oxalic acid (20:80); flow rate 0.50 ml/min; detection, UV 280 nm.

(2) acetonitrile – 0.05% trifluoroacetic acid (TFA) (1:4) containing 0.005 M acetylacetone; flow rate 0.50 mL/min; detection, UV 495 nm.

However, when using the two solvent systems (1) and (2) above, the separation of laccaic acids A from B could not be achieved. Therefore, the mobile phase consisting of 50 mM $NH_4H_2PO_4$ – CH_3OH (85:15); pH 7.8 (adjusting pH with 28% NH_3 solution) at a flow rate of 0.80 mL/min (detection, UV 400 nm) was chosen for analysis because it showed a good separation of laccaic acids A, B and C at the retention times of 4.8, 15.3 and 26.0 minutes respectively as shown in Figure 2.3.

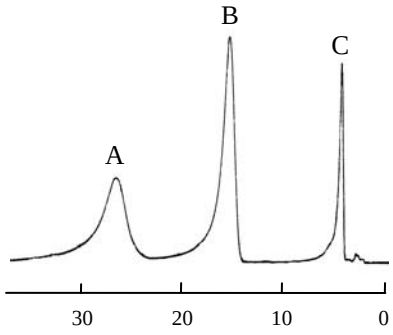


Figure 2.3 HPLC chromatogram of laccaic acids. A, laccaic acid A; B, laccaic acid B and C, laccaic acid C. HPLC conditions: column, Econosil C18 (10 μm, 250 x 4.6 mm i.d.); mobile phase, 50 mM $NH_4H_2PO_4$–CH_3OH (85:15); pH 7.8 (adjusting pH with 28% aqueous NH_3 solution); flow rate of 0.80 mL/min; detection, UV 400 nm.

2.2.7.2 ^{1}H- and ^{13}C-Nuclear Magnetic Resonance (NMR) spectra

All nuclear magnetic resonance (NMR) spectroscopy was performed on a Varian Unity 500 MHz spectrometer. Proton NMR (^{1}H-NMR) spectra and carbon NMR (^{13}C-NMR) spectra were acquired at 500 and 126 MHz respectively. All spectra were recorded in dimethyl sulfoxide (DMSO-d6) with 0.5% tetramethylsilane (TMS), obtained from Cambridge Isotope Laboratories Inc., unless otherwise stated. TMS (0.00 ppm) was used as the internal standard. Chemical shifts (δ) were measured in parts per million (ppm) and referenced against (TMS), and coupling constants (*J*) were measured in hertz (Hz). Superscript letters indicate interchangeable assignments. Multiplicities are denoted as singlet (s), broad single (bs), doublet (d), broad doublet (bd), doublet of doublets (dd), triplet (t), quartet (q) and multiplet (m). The arrangement of ^{1}H-NMR spectral data is listed as: chemical shift followed in brackets

by multiplicity, integration, coupling constant (s) and ascribed assignment. The assignments were made by standard gradient correlation spectroscopy (gCOSY), gradient heteronuclear single quantum correlation (gHSQC), gradient heteronuclear multiple bond correlation (gHMBC) and distortionless enhancement by polarization transfer (DEPT) spectroscopy.

2.2.7.3 Mass spectrometry (MS)

Low resolution (ES) for (MH^-) mass spectrometry was performed on a Micromass LCZ spectrometer. The mass to charge (*m/z*) values of the principal ion peaks are stated with their relative intensities in parentheses. High-resolution (ES) MS for (MH^+) was performed on a Micromass QTof 2 mass spectrometer using a cone voltage of 30V and polyethylene glycol (PEG) as an internal reference. Solvent name abbreviations used are: CH_3CN (acetonitrile), H_2O (water) and MeOH (methanol). The accuracy of the measured HRMS relative to the required molecular weight is given in ppm.

2.3 Results and discussion

2.3.1 Source of stick lac

The percentage of crude extract derived from Thai stick lac from Nakhon Ratchasima and Surin Provinces is given in Table 2.2. The results showed that the percentage crude extract using the same extraction method was similar from both sources. However, the percentage wax removal from Surin Province material was higher than that from Nakhon Ratchasima Province material. The percentage of laccaic acid extract with methanol was found to be 74.8% and 60.1% respectively for

Nakhon Ratchasima and Surin provinces, indicating that the quantity of lac components is dependent upon the locality.

Table 2.2 Effect of source of the stick lac on the yield of extracts from the hot water extraction

Source	**Nakhon Ratchasima stick lac**	**Surin stick lac**
Extraction method	Hot water	Hot water
Extraction time (hour)	1	1
Temperature (^{o}C)	60-75	60-75
% Crude extract	4.3	4.6
% Wax removal	4.5	7.0
% Laccaic acid extract with methanol	74.8	60.1

The percentages of crude extract from Thai stick lac from Surin province by different extraction methods are listed in Table 2.3. It was found that the percentage crude extract using the hot water, ultrasonication and microwave extraction was 4.6, 4.2 and 5.3% respectively. Higher wax content in the crude extract was found using the ultrasonication extraction method. After extracting laccaic acids from the three crude extracts with methanol, the percentage laccaic acid extract via the ultrasonication method was higher than that from the hot water and microwave extraction methods. This can be explained by the fact that the ultrasonication extraction is similar to the situation in a heterogeneous catalytic reaction, which uses

the pores of a solid catalyst under sonication for reaction (Lunhe, 1998). In the vicinity of the stick lac surfaces, asymmetric bubble collapse gives rise to water microjets directed towards the surface. This phenomenon causes erosion, solid breakage and collisions between particles accelerated by the acoustic field (Lunhe, 1998). An increase in water diffusion in and out of the pores of stick lac would result in an increased removal of lac dye from the stick lac. Therefore, the removal of lac dye may be influenced by heat and mass transfer to and from the stick lac and by diffusion in and out of the stick lac pores.

Table 2.3 Effects of the extraction methods at the same stick lac source

Source	**Surin**	**Surin**	**Surin**
Extraction method	Hot water	Ultrasonication	Microwave
Extraction time	1 hour	1 hour	15 min
Temperature (^{o}C)	60-75 ^{o}C	60 ^{o}C	60-70 ^{o}C
% Crude extract	4.6	4.2	5.3
% Wax removal	7.0	7.7	6.0
% Laccaic acid extract with methanol	60.1	69.8	55.6

The results showed that the percentage of the crude extract obtained from the microwave-assisted extraction method was higher than that from the hot water and ultrasonication-assisted methods. The microwave-assisted extraction only needed 15 minutes for full extraction, whereas the hot water and ultrasonication methods needed 1 hour. Due to the considerable saving of time and high extraction efficiency, the

microwave-assisted extraction was more effective than the conventional methods. The percentage of lac dye extracted with methanol was less than that from the other two methods; however, the total percentage laccaic acid after separation by column chromatography was greater than from the other two methods. Microwaves are a form of electromagnetic energy at the lower frequency end of the electromagnetic spectrum (Hayes, 2002). Microwave energy consists of an electric field and a magnetic field, though only the electric field transfers energy to heat a substance without cleaving molecular bonds. Molecular motions by ions and rotation of dipoles take place during the microwave irradiation process (Camel, 2000). Heat generation in the sample in the microwave field requires the presence of a dielectric compound. The effect of microwave energy depends upon the nature of both the solvent and the matrix. In general, the solvent chosen has a high dielectric constant, so that it strongly absorbs the microwave energy (Camel, 2000). To extract lac dye from the stick lac, the water molecules (Dielectric constant = 78.5 (R. J. Fessenden and J. S. Fessenden, 1979)) absorbed the microwave energy leading to localized heating and the temperature increased rapidly to near or above the boiling point of water. This would presumably assist the rupture of stick lac particles and allow the lac dye to flow towards the water and then dissolve. Therefore, the microwave-assisted extraction allowed the migration of the lac dye out of the stick lac thus providing the high extraction rate compared to the hot water and ultrasonication extraction rates. Similar behaviour has been observed in the extraction of triazines from soil samples and the extraction of essential oils from plant materials (Camel, 2000).

2.3.2 Separation of lac dye components

Separation of the lac dye components from the three crude extracts after extraction with methanol was carried out on a cellulose column.

2.3.2.1 Separation of lac dye components from the ultrasonication extract

A typical run from the ultrasonication extract is summarized in Tables 2.4 and 2.5. HPLC chromatograms of the three fractions after purification are shown in Figure 2.4. Two peaks in fraction 1 were detected at the retention times of 14 and 23 minutes respectively. After spiking standard laccaic acid A, the peak area at the retention time of 23 minutes was increased. In a similar way, when standard laccaic acid B was spiked into this fraction, an increase in the peak area at the retention time of 14 minutes was observed. This indicated that fraction 1 contained laccaic acids A and B.

Table 2.4 Chromatographic separation of the crude lac dye from the ultrasonication extract

Fraction	Volume of eluant (mL)	Total amount (g)	Fraction of crude (%)	Component	Eluant
1	2116	0.7100	26.9	Laccaic acids A and B	E:A:W[(a)]
2	200	0.3500	13.3	Laccaic acid C	M:A:W[(b)]
3	900	0.1400	5.3	Laccaic acid C	M:A:W[(b)]

[(a)] E:A:W was the organic phase of ethyl acetate : acetic acid : water (4:1:5).

[(b)] M:A:W was the solvent system of methanol : acetic acid : water (90:5:5).

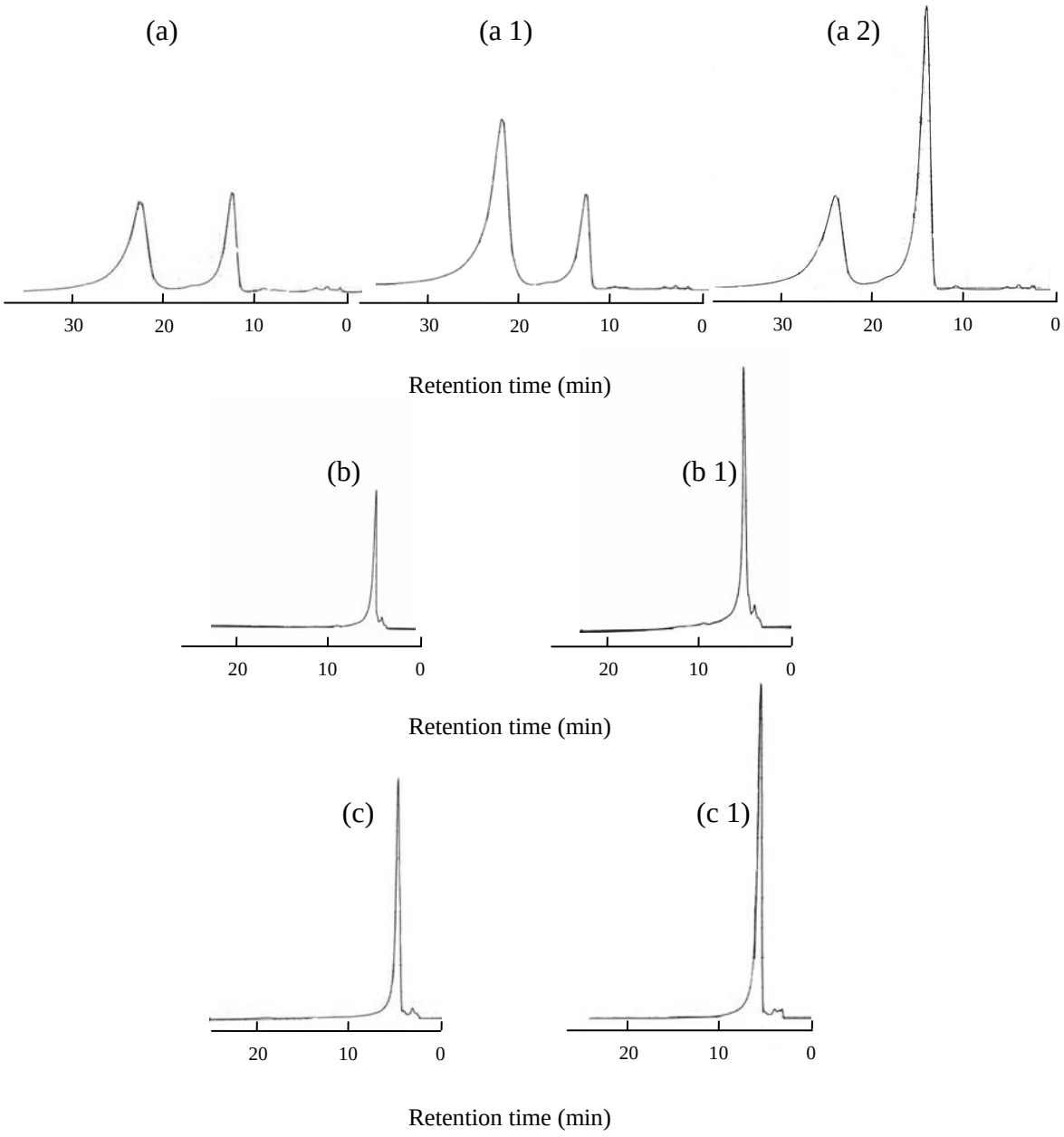


Figure 2.4 HPLC chromatograms of three fractions from the ultrasonication extract: (a) fraction 1; (a 1) fraction 1 spiking with standard laccaic acid A; (a 2) fraction 1 spiking with standard laccaic acid B; (b) fraction 2; (b 1) fraction 2 spiking with standard laccaic acid C; (c) fraction 3; and (c 1) fraction 3 spiking with standard laccaic acid C.

Furthermore, a LRMS (Low Resolution Mass Spectroscopy) (Electrospray; ES) (for MH^-) of this fraction gave molecular ion peaks at *m/z* 536 (100%) $[M-H]^-$ and 495 (70%) $[M-H]^-$ which corresponded to the loss of a hydrogen ion as in laccaic acids A and B respectively as shown in Figure 2.5. Therefore, it could be concluded that fraction 1 contained 60% laccaic acid A and 40% laccaic acid B.

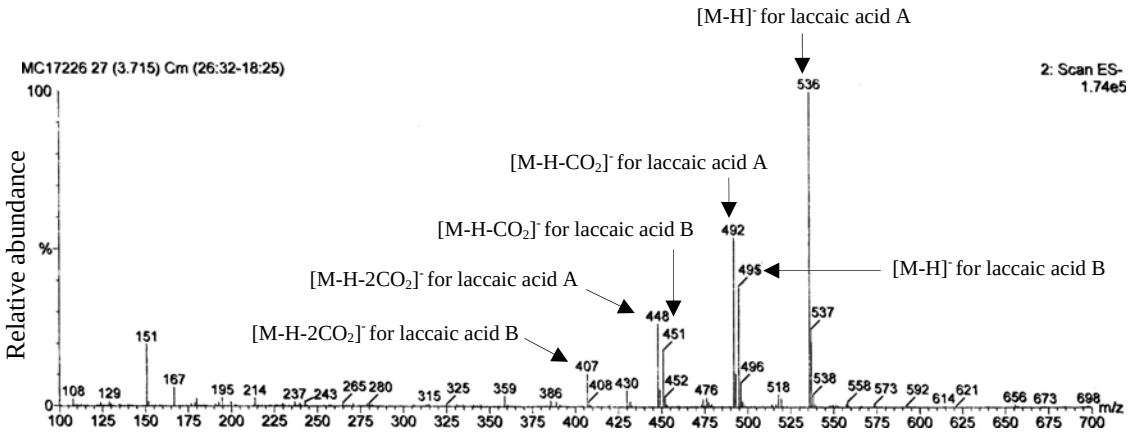


Figure 2.5 Low resolution (ES) mass spectrum for (MH^-) of fraction 1 from the ultrasonication extract.

From 2.63 g of the crude extract from the ultrasonication extraction, the chromatographic separation yielded 64.2 mg of laccaic acid A mixed with 42.8 mg of laccaic acid B (fraction 1) as well as 43.0 mg of laccaic acid C (fractions 2 and 3), as listed in Table 2.5.

Based on the HPLC analysis of fractions 2 and 3, a peak at the retention time of 4.7 minutes was detected in both fractions (Figure 2.4). After spiking standard laccaic acid C, the peak area for the peak at the retention time of 4.7 minutes was increased. The percentage purity of fractions 2 and 3 obtained from HPLC analysis

was 95%. LRMS (ES) (for MH^-) on these fractions also revealed the presence of base peaks in fractions 2 and 3 at *m/z* 538 (100%) [M-H^-] and 538 (100%) [M-H^-] respectively as shown in Figure 2.6. A HRMS (ES) gave an MH^+ peak at *m/z* 540.0779, 0.2 ppm (calculated for $C_{25}H_{18}NO_{13}$ 540.0778) and indicated the most likely molecular formula of the compound in fraction 3 to be $C_{25}H_{17}NO_{13}$.

Table 2.5 Weight of laccaic acids with the percentage purity in each fraction from the ultrasonication extract

Fraction	Component	Weight of laccaic acid (mg)	Percentage purity(%)
1	Laccaic acid A	64.2	60
	Laccaic acid B	42.8	40
2	Laccaic acid C	8.0	95
3	Laccaic acid C	35.0	95

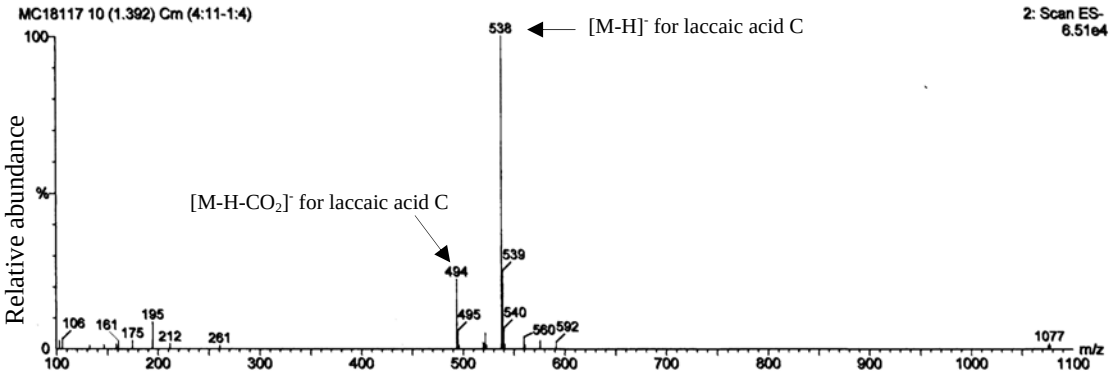
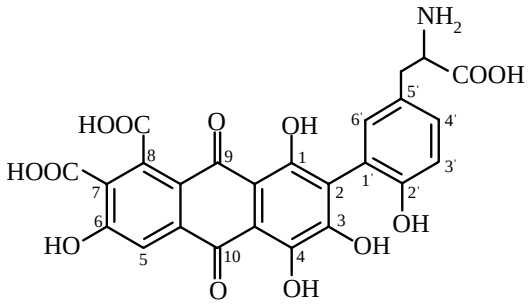


Figure 2.6 Low resolution (ES) mass spectrum for (MH^-) of fraction 3 from the ultrasonication extract.



(1a)

The ^{1}H-NMR spectrum (500 MHz, DMSO-d6) fraction 3 showed the characteristic downfield signal for the carboxylic proton at δ 14.01. There were four signals in the aromatic region at δ 7.51 (s, 1H, H-5), 7.15 (bd, 1H, *J* 8Hz, H-4′), 7.05 (d, 1H, *J* 2 Hz, H-6′) and 6.95 (d, 1H, *J* 8 Hz, H-3′). A broad signal ascribed to a benzylic proton CH_2 at δ 3.10 was also observed. In addition, a broad peak at 4.19 ppm was assigned to a single proton attached to a benzylic in the side chain. Further, spectroscopic investigation by g-COSY spectroscopy revealed a coupling between this broad peak at 4.19 ppm and the ascribed benzylic proton at 3.10 ppm. In addition, it showed a coupling in the aromatic region for H-3′ coupled to H-4′ and H-6′, and a coupling between H-6′ and H-4′. The ^{13}C-NMR spectrum showed 25 carbon atoms which included a benzylic carbon at 36.4 ppm and a single carbon attached to the benzylic carbon at 54.9 ppm. All the evidence supported laccaic acid C **(1a)** as the compound obtained from fraction 3.

2.3.2.2 Separation of lac dye components from the microwave extract

Lac components from the microwave crude extract were isolated using cellulose column chromatography which yielded three fractions as shown in Table 2.6. Each of the three fractions after purification was analyzed using HPLC. HPLC chromatograms of each of the fractions are shown in Figure 2.7. As a result, the HPLC chromatograms of each fraction was likely to be the same as that derived from

the ultrasonication extract, indicating the presence of the same components in both extracts. However, the principal components of the lac dye obtained from the microwave crude extract were laccaic acids A and C.

Table 2.6 Chromatographic separation of the crude lac dye from the microwave extract

Fraction	Volume of eluant (mL)	Total amount (g)	Fraction of crude (%)	Component	Eluant
1	2850	1.62	37.9	Laccaic acid A with trace of laccaic acid B	E:A:W[(a)]
2	195	0.52	14.5	Laccaic acid C	M:A:W[(b)]
3	1000	0.06	1.7	Laccaic acid C	M:A:W[(b)]

[(a)] E:A:W was the organic phase of ethyl acetate : acetic acid : water (4:1:5).

[(b)] M:A:W was the solvent system of methanol : acetic acid : water (90:5:5).

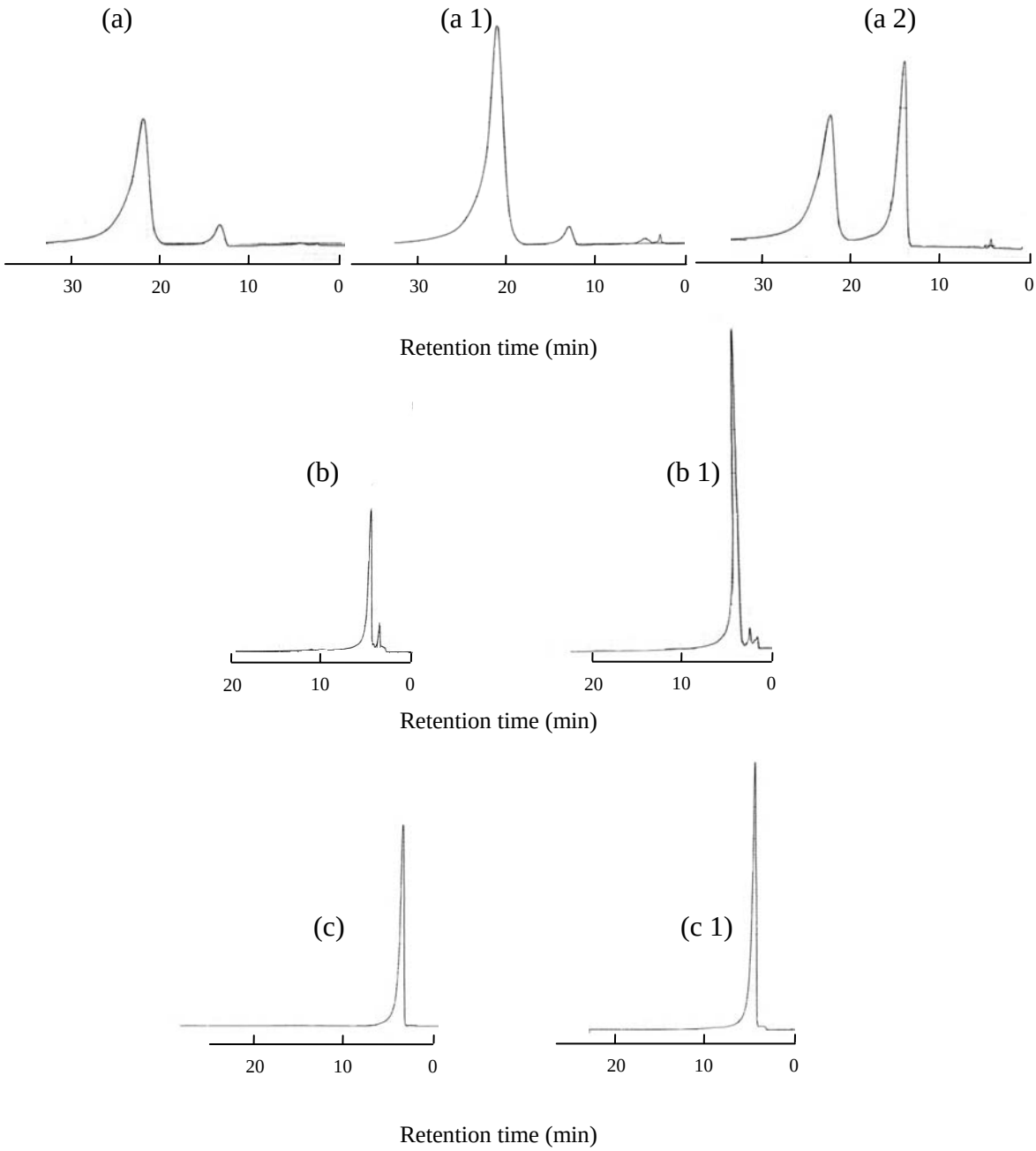


Figure 2.7 HPLC chromatograms of three fractions from the microwave extract: (a) fraction 1; (a 1) fraction 1 spiking with standard laccaic acid A; (a 2) fraction 1 spiking with standard laccaic acid B; (b) fraction 2; (b 1) fraction 2 spiking with standard laccaic acid C; (c) fraction 3; and (c 1) fraction 3 spiking with standard laccaic acid C.

The HPLC chromatogram for fraction 1 showed two peaks at the retention times of 13 and 21 minutes respectively. The peak areas of both peaks increased after spiking with standard laccaic acids A and B into this fraction. This indicated that fraction 1 contained mainly laccaic acid A **(2a)** (95%) with a trace (5%) of laccaic acid B. LRMS (ES) for (MH^-) of laccaic acids A and B in this fraction revealed [$M-H^-$] ions at *m/z* 535.9 (100%) and 495.0 (30%) as shown in Figure 2.8.

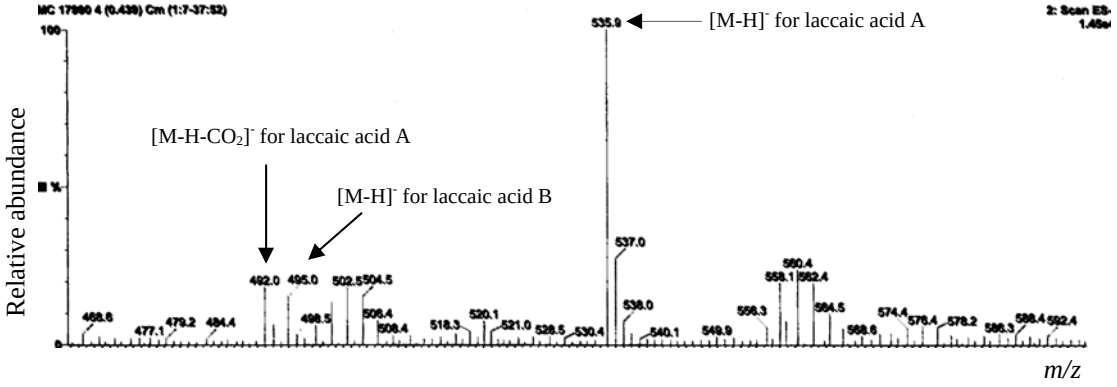
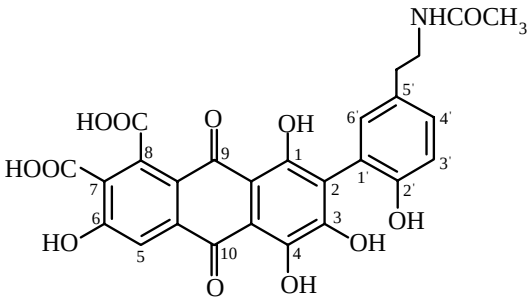


Figure 2.8 Low resolution (ES) mass spectrum for (MH^-) of fraction 1 from the microwave extract.

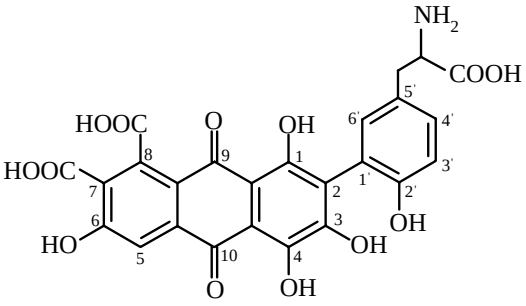


(2a)

The analysis of the ^{1}H-NMR spectrum of this fraction showed that it correlated with the expected downfield carboxylic acid signals (13.53 and 13.16 ppm). The integration of the aromatic region confirmed four aromatic protons at δ 7.77 (s, 1H, H-5), 7.03 (bd, 1H, *J* 8.5 Hz, H-4′), 6.88 (d, 1H, *J* 2 Hz, H-6′) and 6.83 ppm (d, 1H, *J* 8 Hz, H-3′). A sharp singlet

at 1.78 ppm was assigned to the C-methyl group. A two-proton quartet and two-proton triplet at 3.20 (*J* 7 Hz) and 2.61 ppm (*J* 7.5 Hz) representing a CH_2 attached to O or N-CO-, and a benzylic CH_2 respectively, were also observed. A single-proton signal at 7.90 ppm (*J* 5.5 Hz) assigned to an NH proton with a methylene group attached to the NH group was also seen. However, the ^{1}H-NMR spectrum did not clearly show the characteristic signals of the laccaic acid B side chain, due to its low concentration.

A peak at a retention time of 4.7 minutes was detected in the HPLC chromatogram of fractions 2 and 3. It was confirmed that both fractions contained laccaic acid C after spiking with standard laccaic acid C. The percentage purity of the laccaic acid C **(1a)** contained in fractions 2 and 3 was 90% and 100% respectively. Laccaic acid C obtained from fraction 3 was characterized by ^{1}H-NMR spectroscopy, LRMS (ES) (for MH^-), and HRMS (ES) (for MH^+).



(1a)

The ^{1}H-NMR spectrum clearly showed four aromatic protons at δ 7.50 (s, 1H, H-5), 7.03 (bd, 1H, *J* 8 Hz, H-4′), 6.94 (d, 1H, H-6′) and 6.83 ppm (d, 1H, *J* 8 Hz, H-3′). In addition, two broad signals at 3.03 and 4.21 ppm were ascribed to a benzylic proton and a proton attached to the benzylic proton in the side chain. The LRMS (ES) (for MH^-) showed an $[M-H^-]$ ion at *m/z* 538 (100%) as shown in Figure 2.9. The HRMS (ES) gave an ion at *m/z* 540.0786 for MH^+, 1.50 ppm (calculated for $C_{25}H_{18}NO_{13}$ 540.0778) which was supportive of the molecular formula $C_{25}H_{17}NO_{13}$.

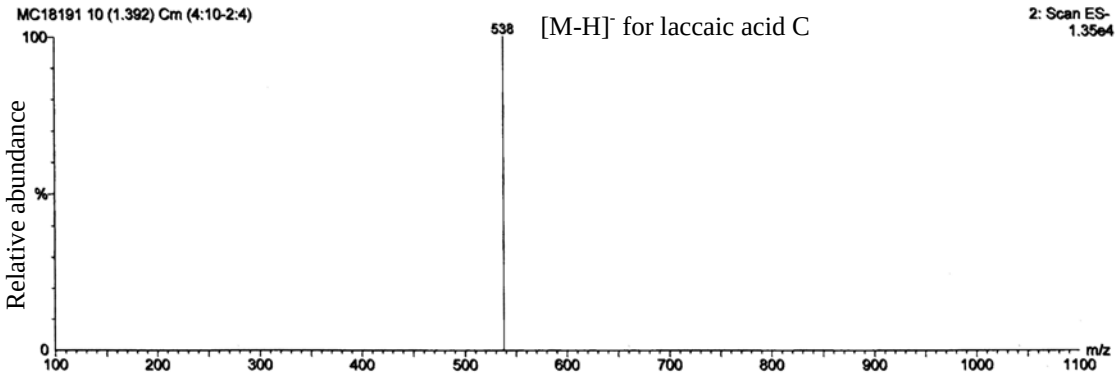


Figure 2.9 Low resolution (ES) mass spectrum for (MH^-) of fraction 3 from the microwave extract.

From 3.59 g of the crude extract, the chromatographic separation yielded 188.9 mg of laccaic acid A mixed with a trace of laccaic acid B (9.9 mg) (fraction 1) as well as 21.0 mg of laccaic acid C (fractions 2 and 3) as given in Table 2.7.

Table 2.7 Weight of laccaic acids with the percentage purity in each fraction from the microwave extract

Fraction	Component	Weight of laccaic acid (mg)	Percentage purity (%)
1	Laccaic acid A	188.9	95
	Laccaic acid B	9.9	5
2	Laccaic acid C	10.0	90
3	Laccaic acid C	11.0	100

2.3.2.3 Separation of lac dye components from the hot water extract

(a) Stick lac from Surin Province

Lac components from the hot water extract were isolated on a cellulose column as listed in Table 2.8. The HPLC chromatograms of the three fractions obtained are shown in Figure 2.10.

Analysis of the HPLC chromatograms of fractions 1 and 3 showed a similar composition to that observed from the ultrasonication and the microwave extracts. The percentage purity of laccaic acids A and B in fraction 1 was 58% and 42% respectively. The LRMS (ES) for (MH^-) confirmed the presence of laccaic acids A (at *m/z* 536, 70%) and B (at *m/z* 495, 43%) in fraction 1 and laccaic acid C in fractions 2 and 3 (Figure 2.11). The ^{1}H-NMR spectrum of the compound from fraction 3 was indicative of that of laccaic acid C. This was further confirmed by HRMS (ES), which gave a molecular ion peak at *m/z* 540.0784 MH^+, 1.00 ppm (calculated for $C_{25}H_{18}NO_{13}$ 540.0778) corresponding to the molecular formula $C_{25}H_{17}NO_{13}$.

Table 2.8 Chromatographic separation of the crude lac dye from the hot water extract

Fraction	Volume of eluant (mL)	Total amount (g)	Fraction of crude (%)	Component	Eluant
1	1268	0.46	45.1	Laccaic acids A and B	E:A:W[(a)]
2	90	0.16	15.7	Laccaic acid C	M:A:W[(b)]
3	770	0.15	14.7	Laccaic acid C	M:A:W[(b)]

[(a)] E:A:W was the organic phase of ethyl acetate : acetic acid : water (4:1:5).

[(b)] M:A:W was the solvent system of methanol : acetic acid : water (90:5:5).

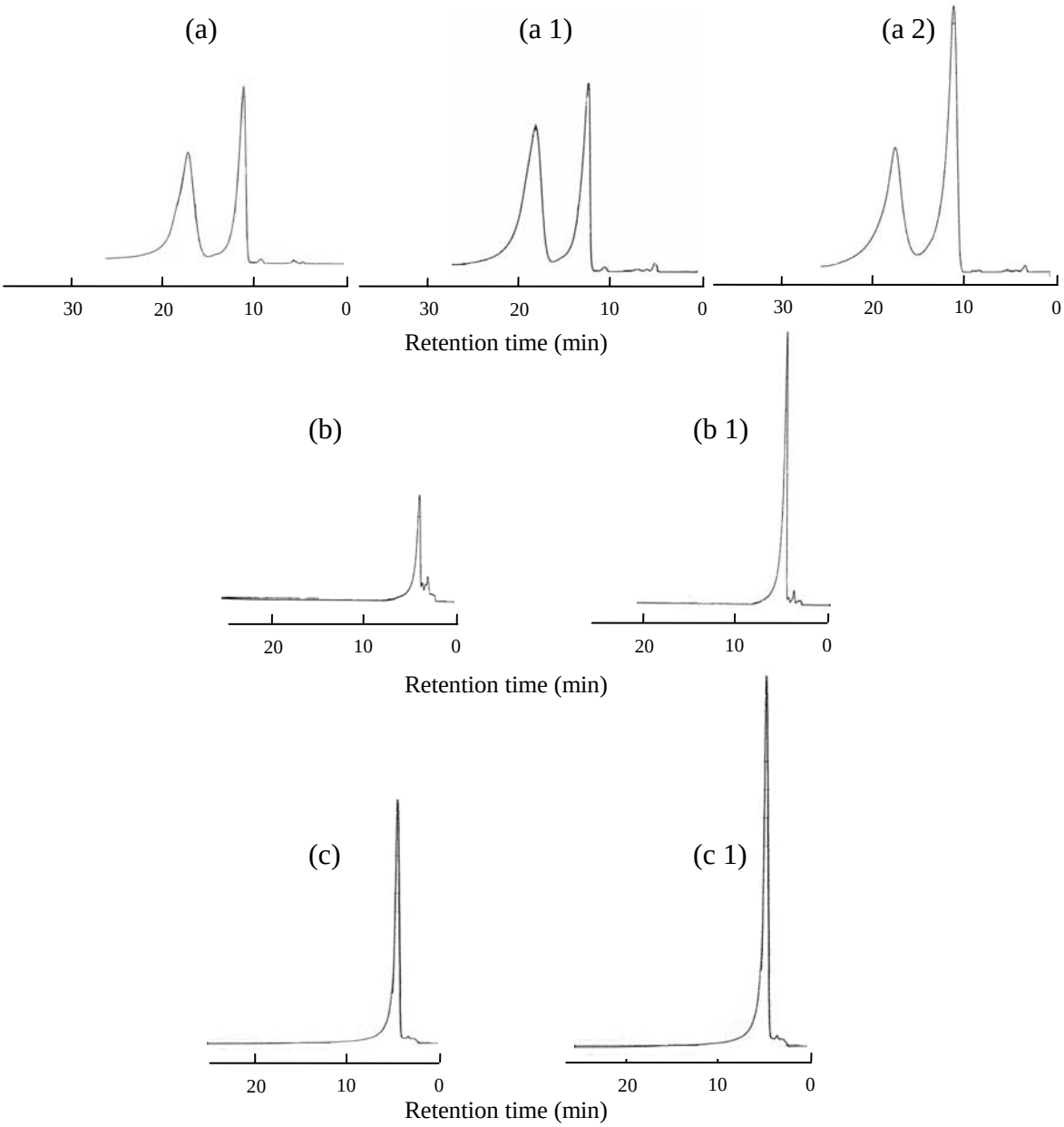


Figure 2.10 HPLC chromatograms of three fractions from the hot water extract (Surin Province): (a) fraction 1; (a 1) fraction 1 spiking with standard laccaic acid A; (a 2) fraction 1 spiking with standard laccaic acid B; (b) fraction 2; (b 1) fraction 2 spiking with standard laccaic acid C; (c) fraction 3; and (c 1) fraction 3 spiking with standard laccaic acid C.

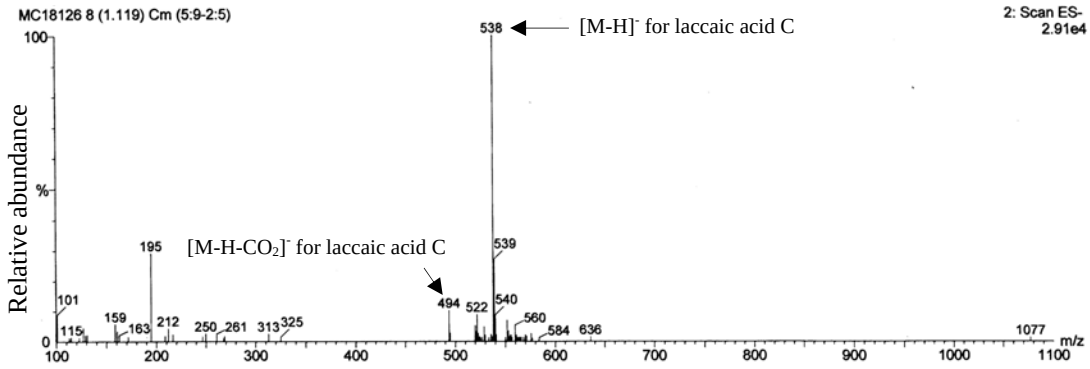


Figure 2.11 Low resolution (ES) mass spectrum for (MH^-) of fraction 3 from the hot water (Surin Province).

From 1.02 g of the crude extract from stick lac from Surin Province, the chromatographic separation gave 20.5 mg of laccaic acid A mixed with 14.9 mg of laccaic acid B (fraction 1) as well as 14.0 mg of laccaic acid C (fractions 2 and 3) as listed in Table 2.9.

Table 2.9 Weight of laccaic acids with the percentage purity in each fraction from the hot water extract

Fraction	Component	Weight of laccaic acid (mg)	Percentage purity (%)
1	Laccaic acid A	20.5	58
	Laccaic acid B	14.9	42
2	Laccaic acid C	1.5	82
3	Laccaic acid C	12.5	95

(b) Stick lac from Nakhon Ratchasima Province

In a similar way to the stick lac extract from Surin Province, a cellulose column was used to separate the lac dye components from stick lac from Nakhon Ratchasima. Table 2.10 shows the chromatographic separation of the crude lac dye from the hot water extract. The obtained fractions were analyzed by HPLC as shown in Figure 2.12.

Table 2.10 Chromatographic separation of the crude lac dye from the hot water extract

Fraction	Volume of eluant (mL)	Total amount (g)	Fraction of crude (%)	Component	Eluant
1	1291	0.39	35.8	Laccaic acid A and trace of B	E:A:W[(a)]
2	102	0.33	30.3	Laccaic acid C	M:A:W[(b)]
3	926	0.10	14.7	Laccaic acid C	M:A:W[(b)]

[(a)] E:A:W was the organic phase of ethyl acetate : acetic acid : water (4:1:5).

[(b)] M:A:W was the solvent system of methanol : acetic acid : water (90:5:5).

Comparison between the HPLC chromatograms of fractions 1 and 3 and the HPLC chromatograms of these fractions from the hot water extract, derived from Surin Province material, showed two peaks in fraction 1 and one peak in fraction 3 at the same retention time. After spiking standard laccaic acids A and B into fraction 1, the peak areas of the peaks at the retention times of 18.0 and 12.0 minutes were

increased. It was found that fraction 1 contained laccaic acids A and B. In similar way, when standard laccaic acid C was spiked into fractions 2 and 3, this led to an increase in the peak area for the peak at the retention time of 4.8 minutes. The LRMS (ES) (for MH^-) confirmed the presence of laccaic acids A (at *m/z* 536, 100%) and B (at *m/z* 495, 30%) in fraction 1 and laccaic acid C in fractions 2 and 3 (Figure 2.13). Structural elucidation based on ^{1}H-NMR spectroscopic data led to the conclusion that the structure of the obtained compound from fraction 3 was consistent with laccaic acid C. Further analysis by HRMS (ES) (at *m/z* measured mass: 540.0782 MH^+, $C_{25}H_{18}NO_{13}$ requires 540.0778, 0.70 ppm) supported the molecular formula of the obtained compound from fraction 3 to be $C_{25}H_{17}NO_{13}$.

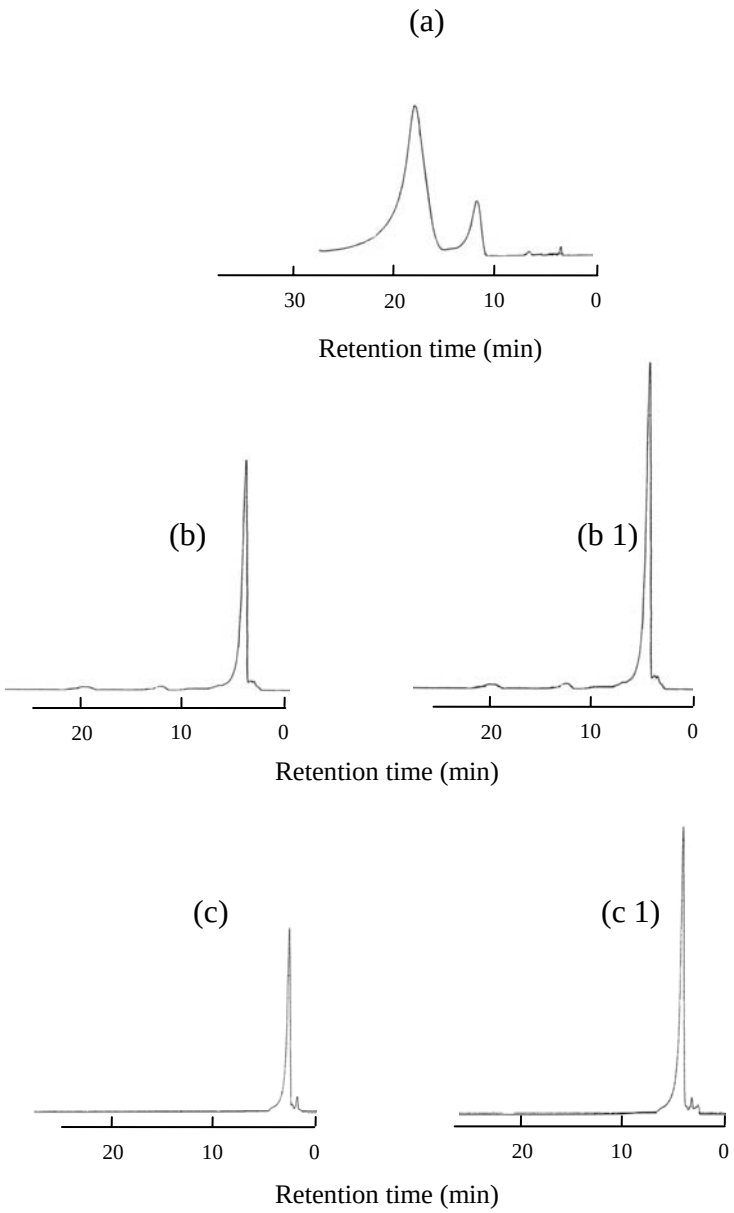


Figure 2.12 HPLC chromatograms of three fractions from the hot water (Nakhon Ratchasima Province) : (a) fraction 1 (b) fraction 2; (b 1) fraction 2 spiking with standard laccaic acid C; (c) fraction 3; and (c 1) fraction 3 spiking with standard laccaic acid C.

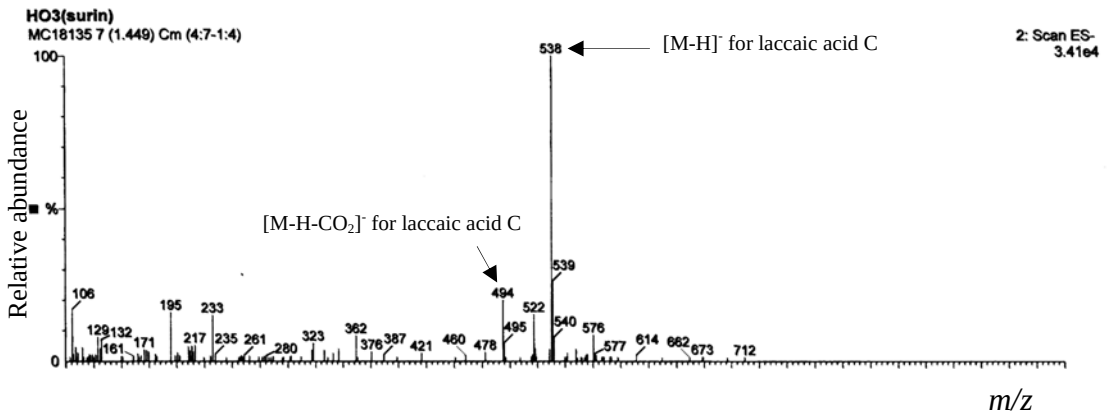


Figure 2.13 Low resolution (ES) mass spectrum for (MH^-) of fraction 3 from the hot water (Nakhon Ratchasima Province).

From 1.09 g of the crude extract from stick lac from Nakhon Ratchasima Province, the chromatographic separation yielded 47.0 mg of laccaic acid A mixed with a trace of laccaic acid B (9.0 mg) (fraction 1) and 16.5 mg of laccaic acid C (fractions 2 and 3) as listed in Table 2.11.

Table 2.11 Weight of laccaic acids with the percentage purity in each fraction from the hot water extract

Fraction	Component	Weight of laccaic acid (mg)	Percentage purity (%)
1	Laccaic acid A	47.0	87
	Laccaic acid B	7.0	13
2	Laccaic acid C	6.5	90
3	Laccaic acid C	10.0	95

The separation results of each lac dye extract with methanol are shown in Table 2.12. The percentage total of laccaic acid from the lac dye extract from Nakhon Ratchasima province extracted with hot water was higher than that from Surin province. Laccaic acid derived from lac dye extract from Nakhon Ratchasima and Surin provinces represented about 6.46% and 4.84% by weight of lac dye extract respectively. Laccaic acid A (4.31%) and laccaic acid C (1.51%) were the major components with a trace of laccaic acid B (0.64%) obtained from lac dye extract from Nakhon Ratchasima material whereas laccaic acids A, B and C were found in the Surin sourced material with percentage of 2.01, 1.46 and 1.37% respectively. This indicated that the quantity and composition of lac components are dependent upon the source of stick lac (Yamada *et al*., 1989; Oka, *et al*., 1998).

The total percentage of laccaic acid represented about 4.84, 5.70 and 6.12% by weight of lac dye extract by using the hot water, ultrasonication and microwave extraction methods respectively as listed in Table 2.12. The total percentage laccaic acid via the ultrasonication and microwave extraction methods were higher than that from the hot water extraction method. It was shown that the ultrasonication and microwave extract methods increased the solubility of lac dye in water. As also reported by other workers (Burwood *et al*., 1965; Oka *et al*., 1998), the major component obtained from the three extraction methods by column chromatography was laccaic acid A with the minor laccaic acids being B and C, especially from the microwave extraction. In addition, the percentage of laccaic acid B obtained by the hot water and ultrasonication extraction methods were essentially the same.

Table 2.12 A summary of the separation results in each crude extract of the stick lac from Surin and Nakhon Ratchasima Provinces

	Crude extract											
	Hot water			**Hot water**			**Ultrasonication**			**Microwave**		
Source of stick lac	Korat			Surin			Surin			Surin		
Extraction time	1 hour			1 hour			1 hour			15 min		
Temperature	60-75 ^{o}C			60-75 ^{o}C			60 ^{o}C			60-70 ^{o}C		
% crude extract	4.3			4.6			4.2			5.3		
% lac dye after removing wax	95.5			93.0			92.3			94.0		
% wax	4.5			7.0			7.7			6.0		
% lac dye extract	74.8			60.1			69.8			55.6		
Wt. lac extract for column (g)	1.09			1.02			2.63			3.59		
Wt. of cellulose (g)	60			60			160			160		
Fraction	HK1	HK2	HK3	SU1	SU2	SU3	UL1	UL2	UL3	MI1	MI2	MI3
Solvent	E:A:W	M:A:W	M:A:W	E:A:W	M:A:W	M:A:W	E:A:W	M:A:W	M:A:W	E:A:W	M:A:W	M:A:W
Volume (mL)	1291	120	926	1268	90	770	2116	200	900	2850	195	1000
Wt. of fraction (g)	0.39	0.33	0.10	0.46	0.16	0.15	0.71	0.35	0.14	1.62	0.52	0.06
% yield	35.8	30.3	14.7	45.1	15.7	14.7	26.9	13.3	5.3	37.9	14.5	1.7
HPLC	A+B	C	C	A+B	C	C	A+B	C	C	A+B	C	C
(after purification)	87:13%	90%	95%	58:42%	82%	95%	60:40%	95%	95%	95:5%	90%	100%
Retention time (min)	18.0:12.0	4.8	4.8	18.6:11.5	4.8	4.8	23.4:14.2	4.7	4.7	20.6:13.8	4.6	4.8
ESMS^{-}	A+B	C	C	A+B	C	C	A+B	C	C	A+B	C	C
Wt. of laccaic acid after purification (mg)	47.0:7.0	6.5	10.0	20.5:14.9	1.5	12.5	64.2:42.8	8.0	35.0	188.9:9.9	10.0	11.0
HRMS (ES^{-})	-	-	C	-	-	C	-	-	C	-	-	C
NMR (^{1}H and ^{13}C-NMR)	-	-	C	-	-	C	A+B	-	C	A	-	C
Laccaic acid A:B:C (mg)	47.0:7.0:16.5			20.5:14.9:14.0			64.2:42.8:43.0			188.9:9.9:21.0		
% laccaic acid A:B:C	4.31:0.64:1.51			2.01:1.46:1.37			2.44:1.63:1.63			5.26:0.28:0.58		
% total of laccaic acid (by weight of extract)	6.47			4.84			5.70			6.12		

2.4 Conclusions

Ultrasonication and microwave extraction methods were used to extract lac dye from the Thai stick lac from Surin province and these were compared with the hot water extraction method. It was found that the total percentage of laccaic acid obtained by the ultrasonication and the microwave-assisted extraction methods was higher than that from the hot water extraction method. This can be explained by the erosion, solid breakage and collisions between stick lac particles being accelerated by the acoustic field from the ultrasonic bath (Luche, 1998). This phenomenon results in an increased water solubility of lac dye. Due to the considerable saving in time, the microwave-assisted extraction method was more effective than the ultrasonication-assisted and hot water extraction methods. It was found that the microwave extraction method gave the highest total percentage of laccaic acid (6.12% by weight of the lac dye extract). The microwave irradiation seemed to facilitate the migration of laccaic acid out of the stick lac. These results were not substantial; however a slight improvement in the percentage laccaic acid obtained was observed using the new extraction methods (the ultrasonication and microwave-assisted extraction methods) previously unreported in the literature, compared to the traditional extraction method (hot water). However, no new minor components in the lac dye were detected on using these new extraction methods.

The total percentage of laccaic acid obtained from Thai stick lac from Nakhon Ratchasima and Surin provinces was 6.47% and 4.84% respectively, consistent with regional variations in the quantity and composition of lac components.

CHAPTER III

ADSORPTION KINETIC AND THERMODYNAMIC STUDY OF LAC DYEING ON SILK

3.1 Introduction

Silk is a protein-based fibre produced by the silkworm, *Bombyx mori* for the construction of their cocoon (Horrocks and Anand, 2000). Silk fibres are made up of a protein called fibroin. This protein is constructed from layers of anti-parallel beta pleated sheets which run parallel to the silk fibre axis. Each chain of fibroin is made up of multiple repeats of the sequence $(Gly\text{-}Ser\text{-}Gly\text{-}Ala\text{-}Gly\text{-}Ala)_n$, where Gly, Ala and Ser refer to glycine, alanine and serine respectively (Kaplan *et al*., 1994). Like wool, silk fibres contain carboxyl and protonated amino groups which result in its amphoteric characteristics (Carr, 1995). Under acid conditions the carboxyl groups are unionized, leaving a net positive charge which enables dye anions to be adsorbed (Carr, 1995). Many studies have been undertaken to investigate silk dyeing. For instance, the dyeing behaviour of tannic acid treated silk fibre has been reported (Kawahara, 1999). It was found that the pretreatment of silk fibres with 2% tannic acid enhanced the dye exhaustion. This is due to the fact that tannic acid can form complexes with acid dyes. In addition, some anthraquinone violet acid dyes obtained from synthetic sources have been used to dye silk (Zhang, Hou and Tan, 1997). It was found that the dyed silk showed excellent fastness properties.

To understand the silk dyeing process, the kinetic and thermodynamics of dyeing have been studied. According to Giorgi, Colonna and Bianchi (1991), the thermodynamic affinity of acid dyes on silk was investigated. It was found that the dyeing mechanism of silk with acid dyes is likely to be similar to that of wool. Bruce and Broadwood (2000) have also studied the kinetics of wool dyeing with acid dye. They found that the uptake rate of the acid dye is likely to be a second order reaction. The kinetics can be explained in terms of the rate controlling step, which in this case is the reaction between the dye anion and the attachment site, rather than diffusion of the dye to the attachment site. In addition, the correlation between the dyeing rate and thermodynamic affinity of disperse dyes has been reported by Giorgi (1989). It was shown that the quantity of the dye fixed in the alkaline dye bath was related to the quantity adsorbed in the dye bath by the fibre.

Lac dye is classified into the acid dye group based on its application method (Saxena *et al*., 1997). It is applied to dye protein fibres such as silk and wool and polyester (Lokhande, Dorugade and Naik, 1998). In Thailand, lac dye has been used as a natural dyestuff for silk and cotton dyeing for a long time (Moeyes, 1993). The application of lac dyeing on fibres with and without mordants has been reported by several researchers. According to Roy and Pathak (1971a), lac dye was converted into a direct dye by heating with primary aromatic amines, giving 1,4-diarylamino-2-aceto-3-ethyl-6-hydroxy-7,8-dicarboxyarylimido-9,10-anthraquinone. This lac dye-amine condensation product was then sulfonated with oleum to yield the water-soluble trisulfonic acid dye derivative and applied to dye silk fabrics. It was shown that the dyed silk were fast to washing and fast against light. Moreover, the lac dye-amine condensation product was reacted with resorcinol and phenol to give xanthene

derivatives (Roy and Pathak, 1971b). These xanthene derivatives were then sulfonated with oleum giving hexabasic acids and used to dye silk. It was found that different shades of the dyed silk were obtained. Taeko and Keiko (1980) used tin (II) chloride as a mordant for silk dyeing with lac dye [60687-93-6]. It was found that the dyed silk had a red shade and was fast to light and dry cleaning. Faruq, Khan, Alam and Rahman (1991) used lac dye in the dyeing process in the presence of mordants. Alum, copper sulfate, ferrous sulfate and tin chloride were all used as mordants and were applied in dyeing process in order to improve the fastness properties. It was found that the dyed silk had various shades. Dyeing of silk using lac dye obtained from a shellac factory has also been reported by Patra (1998). Different mordants of about 2% to 5% by weight of the material have been applied to treat silk fabric before dyeing. They reported that silk fabric was fast to washing and light after it was treated with mordants. In addition, Lokhande *et al*. (1998) indicated that mordanting was necessary for the dyeing of polyester fibres with lac dye to produce different shades and tones on the polyester by using various mordants. The application of lac dye on tasar silk with respect to degumming, bleaching and dyeing conditions was studied (Ghosh, Sreenivasa, Sengupta and Thangavelu, 2002). Different mordants were used in this study, resulting in various shades of colour on the dyed silk. The effect of mordanting methods and dyeing conditions in the lac dyeing process on the properties of silk yarn was studied (Kongkachuichay, Shitangkoon and Chinwongamorn, 2002). There are three mordanting methods used, pre-mordanting, simultaneous mordanting and post-mordanting, in this study. Memecylon, tamarind, a mixture of memecylon and tamarind (1:1 v/v), formic acid, acetic acid, tartaric acid and alum were used as natural mordants and chemical mordants. As a result, the type of mordant had an

influence on the properties of finished product including fastness properties, colour shade and breaking strength of the silk yarn.

In the north and the northeast of Thailand, lac dye is used as a natural red dyestuff for cotton and silk dyeing but the fastness properties and reproducibility to give consistency in production are still problems to be solved. As part of the approach to tackle these problems, fundamental physical studies on the dyeing process are important.

In previous work, the thermodynamics of adsorption of laccaic acids on silk have been studied (Kongkachuichay *et al*., 2002), but without pH control and the results indicated that the adsorption isotherm of silk dyeing with laccaic acids, from which the erythrolaccin had been removed, was of the Langmuir type. The values for the heat and entropy of dyeing were also reported (Kongkachuichay *et al*., 2002). However, there have not been any complementary studies on the kinetics of the dyeing process. Therefore, the adsorption of lac dye on silk yarn will be measured and determined quantitatively in relation to dye solution pH values, contact time, initial dye concentration, and material to liquor ratio (MLR) in this research. In addition, the Langmuir and Freundlich equations will be used to fit the equilibria of lac dyeing on silk yarn. The scientific results from this study will hopefully give a better understanding of the adsorption mechanism in this dyeing process.

3.1.1 Physical chemistry of dyeing process

The scientific investigation of the dyeing processes involves two experimental methods which can be described by dyeing kinetics and dyeing equilibria (Zollinger, 1991). The dyeing process involves the distribution of a dye between at least two phases, namely dye bath and substrate. The distribution process

is called adsorption if the substance which is to be distributed is retained by a surface (e.g. gas on a solid). If the substance does not stay at the surface but enters the interior of a body (e.g. gas in liquid), the process is termed sorption. Dyeing processes of water soluble dyes in aqueous dye baths with any substrate always requires a distribution process between two phases (dye bath and substrate) (Perkins, 1996). The kinetics of dyeing are represented by dye uptake curves which give the rate of transfer of dye in solution from the dye bath into the substrate. The position of sorption versus desorption after infinite time is represented by the dyeing equilibria of the dyeing process. Graphical representations of a dyeing process are shown in Figure 3.1, by the dye uptake curves (left hand side) and dyeing isotherms (right hand side).

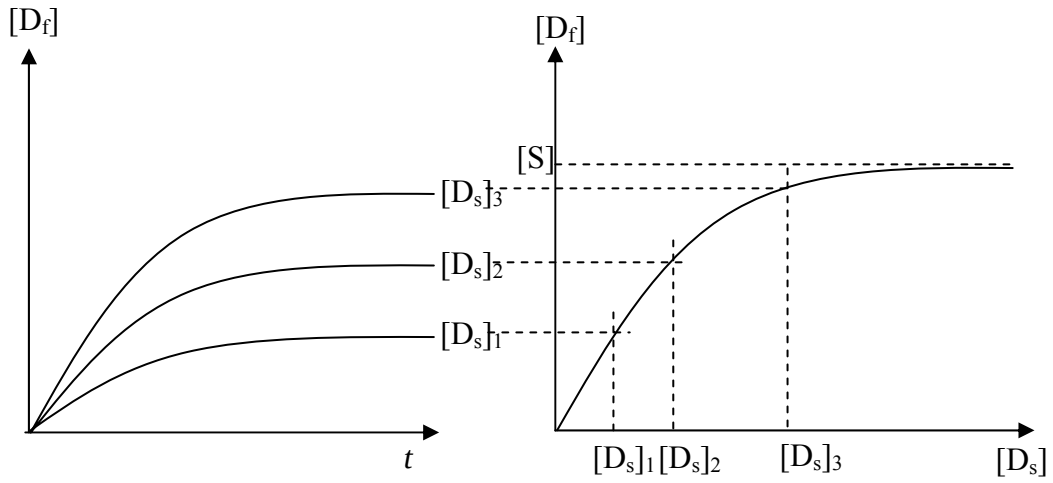


Figure 3.1 Graphical representation of a dyeing process: kinetics (left) and equilibrium (right) (Zollinger, 1991).

$[D_s]$: concentration of dye in solution $[D_f]$: concentration of dye in substrate

t: time [S]: saturation value

If the dyeing process was done under different isothermal conditions, a series of curves (Perkins, 1996) are given, as shown in Figure 3.2. The initial adsorption rate increases with an increasing in dyeing temperature. This can be explained by the fact that the dye adsorption by fibres at higher temperature is faster than that at lower temperature and hence leads to increase the initial rate constant. The slope of curve varies depending on the temperature, type of fibre, type of dye, amount of agitation of the dye bath, amount and type of dyeing auxiliaries used and other factors. As the

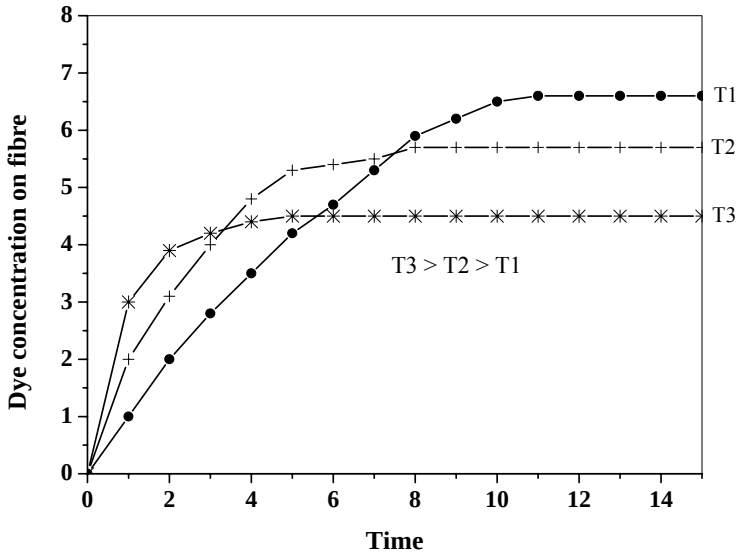


Figure 3.2 Rate of dyeing isotherms (Perkins, 1996).

amount of dye on the fibres increases, the sites being covered, and as a result the dye must leave the surface and diffuse toward the interior of the fibre before additional dye molecules can be adsorbed from the dye bath. The kinetic behaviour of a dye in the dyeing of a textile material comprises at least four stages (Zollinger, 1991):

(a) convectional diffusion to the fibre surface, occurring in the dye bath;

(b) molecular diffusion through the hydrodynamic boundary layer;

(c) adsorption at the outer surface;

(d) molecular diffusion into the fibre (sorption).

The stages (a), (c) and (d) are important for the kinetics of the overall dyeing process. After some period of time, the slope of the isotherm becomes flat indicating that the system has reached equilibrium. The time required to reach equilibrium is always shorter at higher dyeing temperature. It is mainly due to the fibres containing more dye at higher temperatures in the early stages but less dye in the latter stages of dyeing. Therefore, an increasing in the temperature leads to an increase in the dyeing rate but a decrease in the ultimate exhaustion after the equilibrium time, as shown in Figure 3.2. This shows that dye molecules can adsorb to a greater degree at a lower temperature because the dyeing reaction is exothermic.

$$\text{Dye} + \text{Fibre} \rightleftharpoons \text{Dye} — \text{Fibre} + \text{heat}$$

Desorption of dye molecules from fibres to dye bath takes place at higher dyeing temperatures because of more heat in the dyeing process. Thus, the equilibrium position of this process is shifted to the left hand side.

There are several different intermolecular and intramolecular interactions that are essential for understanding the chemistry of textile dyeing. The four main types of

interactions are ionic forces, Van der Waal's forces, hydrogen bonds and dipole-dipole interactions (Zollinger, 1991; Christie *et al*., 2000) and will be described below:

Ionic forces

An ionic bond is formed by electron transfer from one atom to another atom or atoms (R. J. Fessenden and J. S. Fessenden, 1979). The atom that loses electrons becomes a positive-charged species or cation. The atom that gains the electrons becomes a negative-charged species or anion. Ionic bonds are the resulting electrostatic attraction between these oppositely charged ions. In the dyeing of wool, silk and polyamides with anionic dyes, these fibres contain amino and carboxyl groups. Depending on the pH value in dyeing process, under lower pH these fibres have an overall positive charge (NH_3^+) because the carboxyl groups in the side chains are hardly ionized under acid condition. Therefore, anionic dyes are attracted towards the positively charged amino acid by ionic forces.

Van der Waal's forces

Van der Waal's forces or dispersion forces are the forces of attraction involved between non-polar molecules (Christie, 2000). The distances between molecules have an important effect on the strength of van der Waals forces. They are the weakest intermolecular forces. Although in a non-polar molecule there is no overall charge distribution, the electrons are in constant motion so that at any instant, small dipoles will be present. These instantaneous dipoles in turn induce oppositely-oriented dipoles in neighbouring molecules and a weak attraction between the molecules results. Van der Waals forces are therefore only effective for sorption of dyes to fibre molecules if the distance between the dye and the fibre molecules is small. The influence of Van der Waal's forces particularly important in dyeing of cellulosic fibres.

Hydrogen bonding

Hydrogen bonding is an especially strong type of dipole-dipole interactions. This interaction occurs between molecules containing a hydrogen atom which can interact with a nitrogen, oxygen, or fluorine atom (Christie, 2000). A partially positive hydrogen atom of one molecule is attracted to the unshared pair of electrons of the electronegative atom of another molecule. Intermolecular and intramolecular hydrogen bonding between function groups on dye molecules and fibre molecules play important role in many interaction dye-fibre systems.

Covalent forces

Covalent forces result from the sharing of a pair of electrons by two atoms. An especially important use of covalent bonding in colour application technology is the application of reactive dyes to cellulosic and protein textile fibres (Christie, 2000). After dyeing fibres with reactive dyes, the dyes interact chemically with the fibres to form a covalent bond.

Dipole-dipole interactions

Dipole-dipole interactions may make a contribution to the forces of attraction between dye and fibre molecules (Christie, 2000). Dipolar intermolecular forces involve the attraction of the positive centers in one polar molecule for the negative centers in another. As a result of these forces, polar molecules are generally attracted to each other more strongly than are non-polar molecules of comparable molecular size. Non-polar molecules are attracted to each other by weak dipole-dipole interactions called London forces. London forces arise from dipoles induced in one molecule by another so a weak attraction between the molecules results. However, as the charges are only partial, dipolar-dipole interactions are weaker than ionic forces.

3.1.2 Kinetic models of adsorption

In order to investigate the controlling mechanism adsorption processes such as mass transfer, diffusion control and chemical reaction, several methods have been used to test the experimental data. A simple kinetic analysis of adsorption is the Lagergren equation. The Lagergren equation, a pseudo first order equation, describes the kinetics of the adsorption process as follows (Ho and McKay, 1998; Dogan and Alkan, 2002; Sun and Yang; 2002; Chiou, Ho and Li, 2004):

$$\frac{\mathrm{d}\,q_t}{\mathrm{d}\,t} = k_1(q_e - q_t) \qquad (3.1)$$

where k_1 is the rate constant of pseudo first-order adsorption (s^{-1}), and q_e and q_t are the amount of dye adsorbed per gram silk (mg/g silk) at equilibrium and time t. In many cases, the first order equation of Lagergren does not fit well for the whole range of contact times and is generally applicable over only the initial stage of the adsorption (Chiou and Li, 2002; Ozacar and Sengil, 2003). After definite integration by applying the initial conditions $q_t = 0$ at $t = 0$ and $q = q_t$ at $t = t$, equation (3.1) becomes

$$\ln(q_e - q_t) = \ln q_e - k_1 t \qquad (3.2)$$

A straight line of ln (q_e - q_t) versus t suggests the applicability of this kinetic model to fit the experimental data. The first-order rate constant k_1 and equilibrium adsorption density q_e were calculated from the slope and intercept of this line.

In addition, the pseudo second order kinetic model (Ho and McKay, 1998; Wu, Tseng and Juang, 2000; Chiou and Li, 2002) is based on adsorption equilibrium capacity and can be expressed as:

$$\frac{\mathrm{d}\,q_t}{\mathrm{d}\,t} = k_2(q_e - q_t)^2 \qquad (3.3)$$

where k_2 (g silk/mg min) is the rate constant for pseudo second-order adsorption. Integrating equation (3.3) and applying the initial conditions gives:

$$\frac{1}{(q_e - q_t)} = \frac{1}{q_e} + k_2 t \qquad (3.4)$$

or equivalently,

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} t \qquad (3.5)$$

and

$$h_i = k_2 q_e^2 \qquad (3.6)$$

where h_i (Chiou and Li, 2003) is the initial dye adsorption rate (mg/g silk min). If the pseudo second order kinetics are applicable, the plot of t/q_t versus t show a linear relationship. The slope and intercept of (t/q_t) versus t were used to calculate the

pseudo second-order rate constant k_2 and q_e. It is likely that the behaviour over the whole range of adsorption is in agreement with the chemisorption mechanism being the rate-controlling step (Chiou and Li, 2002).

In general, the rates of chemical reactions increase with an increase in the temperature. In the rate law, temperature dependence appears in the rate constant. The dependence of rate constants on temperature over a limited range can usually be represented by an empirical equation proposed by Arrhenius in 1889 (Alberty and Silbey, 1997):

$$k = Ae^{-E_a / RT} \tag{3.7}$$

where A is the pre-exponential factor and E_a is the activation energy. The pre-exponential factor A has the same units as the rate constant. An alternative form is obtained by taking the logarithm of each side.

$$\ln k = \ln A - \frac{E_a}{RT} \tag{3.8}$$

A straight line is obtained by plotting of the logarithm of the rate constant against with the reciprocal of the absolute temperature. Such a graph is often referred to as an Arrhenius plot.

The enthalpy ($\Delta H^{\#}$), entropy ($\Delta S^{\#}$) and free energy ($\Delta G^{\#}$) of activation can be also calculated using the Eyring equation (Laidler and Meiser, 1999) as follows:

$$\ln\left(\frac{k}{T}\right) = \ln\left(\frac{k_b}{h}\right) + \frac{\Delta S^{\#}}{R} - \frac{\Delta H^{\#}}{RT} \qquad (3.9)$$

where k_b and h refer to Boltzmann's constant and Planck's constant respectively. The enthalpy ($\Delta H^{\#}$) and entropy ($\Delta S^{\#}$) of activation were calculated from the slope and intercept of a plot of ln (k/T) versus $1/T$. Gibbs energy of activation ($\Delta G^{\#}$) can be written in terms of enthalpy and entropy of activation (Laidler and Meiser, 1999):

$$\Delta G^{\#} = \Delta H^{\#} - T\Delta S^{\#} \qquad (3.10)$$

3.1.3 Adsorption isotherms

3.1.3.1 The Langmuir isotherm

The equilibrium adsorption isotherm is fundamental in describing the interaction behaviour between solutes and adsorbents, and is important in the design of an adsorption system. The Langmuir adsorption isotherm has been successfully applied to many other real sorption processes (Choy, McKay and Porter, 1999; Chiou and Li, 2002). A basic assumption of the Langmuir theory is that sorption takes place at specific homogeneous sites within the adsorbent. It is then assumed that once a dye molecule occupies a site, no further adsorption can take place at that site. Theoretically, therefore, a saturation value is reached beyond which no further sorption can take place. The saturated monolayer curve can be represented by the expression:

$$q_e = \frac{QbC_e}{1 + bC_e} \tag{3.11}$$

A linear form of this expression is:

$$\frac{C_e}{q_e} = \frac{1}{Qb} + \left(\frac{1}{Q}\right) C_e \tag{3.12}$$

For lower concentrations, the following form of Langmuir equation is found to be more satisfactory (Bhattacharyya and Sarma, 2003):

$$\frac{1}{q_e} = \frac{1}{Q} + \frac{1}{QbC_e} \tag{3.13}$$

In the above equation, Q is the maximum amount of the dye per unit weight of fibre to form a complete monolayer coverage on the surface bound at high equilibrium dye concentration C_e, q_e is the amount of dye adsorbed per units weight of fibre at equilibrium, and b the Langmuir constant related to the affinity of binding sites. The value of Q represents a practical limiting adsorption capacity when the surface is fully covered with dye molecules and assists in the comparison of adsorption performance (Chiou and Li, 2002). The values of Q and b are calculated from the intercepts and slopes of the straight lines of plot of $1/q_e$ versus $1/C_e$.

The essential characteristics of the Langmuir isotherm can be expressed in terms of the dimensionless constant separation factor for equilibrium parameter, R_L

(Sivaraj, Namasivayam and Kadirvelu, 2001; Kannan and Sundaram, 2002; Jain, Gupta, Bhatnagar and Suhas, 2003), defined as follows:

$$R_{\mathrm{L}} = \frac{1}{1 + bC_o} \qquad (3.14)$$

where C_o is the initial concentration of dye (in ppm or mg/L) and b is the Langmuir constant (L/mg). The values of R_{L} indicates the type of isotherm to be irreversible (R_{L}= 0), favourable (0 < R_{L} < 1), linear (R_{L} = 1) or unfavourable (R_{L} > 1).

3.1.3.2 The Freundlich isotherm

The Freundlich isotherm (Choy *et al*., 1999; Chiou and Li, 2002) is a special case for heterogeneous surface energy in which the energy in the Langmuir equation varies as a function of surface coverage strictly due to variation of the sorption. The Freundlich equation is given as:

$$q_e = Q_f C_e^{1/n} \qquad (3.15)$$

where Q_f is roughly an indicator of the adsorption capacity and 1/n of the adsorption intensity. A linear form of the Freundlich expression in the equation (3.15) will yield the constants Q_f and 1/n.

$$\ln q_e = \ln Q_f + \frac{1}{n} \ln C_e \qquad (3.16)$$

Therefore, Q_f and 1/n can be determined from the linear plot of ln q_e versus ln C_e. The magnitude of the exponent 1/n gives an indication of the favourability of adsorption. Values of $n > 1$ obtained represent favourable adsorption conditions (Chiou and Li, 2002).

The thermodynamic parameters for the adsorption process, namely Gibbs energy (ΔG^o), enthalpy (ΔH^o) and entropy (ΔS^o) of adsorption are evaluated using the following equations (Chiou and Li, 2003):

$$K_C = \frac{C_{ad,e}}{C_e} \tag{3.17}$$

$$\Delta G^o = -RT\ln K_c \tag{3.18}$$

$$\ln K_C = \frac{\Delta S^o}{R} - \frac{\Delta H^o}{RT} \tag{3.19}$$

In the above equations, K_c is the equilibrium constant, and $C_{ad,e}$ and C_e are the dye concentration adsorbed at equilibrium (mg/L) and the concentration of dye left in the dye bath at equilibrium (mg/L), respectively. T is the solution temperature (K) and R is the gas constant. Enthalpy (ΔH^o) and entropy (ΔS^o) of the adsorption are calculated from the slope and intercept of van't Hoff plots of ln K_c versus 1/T.

3.2 Experimental

3.2.1 Chemicals and materials

(a) Silk yarn from Pungthungchai, Nakhon Ratchasima Province, Thailand

(b) Standard lac dye [124-04012], standard grade, Wako, Japan

(c) Stick lac from Nong-Ying shop, Muang District, Surin Province, Thailand

(d) Glacial acetic acid, CH_3COOH, Merck, England

(e) Soap, commercial grade, Thailand

(f) Sodium silicate, commercial grade, Thailand

(g) Sodium carbonate, Merck, Germany

(h) Hydrogen peroxide 40% (w/v), commercial grade, Thailand

(i) Hydrochloric acid 37% (w/v), HCl, Merck, Germany

(j) Ethanol 95% (w/v), analytical grade, Merck, Germany

(k) Chitosan, medium molecular weight, viscosity 200.000 cps,
CAS 9012-76-4, FW 161), Aldrich Chemical Company, Germany

3.2.2 Instruments

(a) A Cary 1E UV-Visible spectrophotometer was employed for absorbance measurements using quartz cells of path length 1 cm.

(b) A pH meter (Mettler Delta 320, UK) was used to measure the pH values of the lac dye solutions.

(c) A thermostatted shaker bath (Heto-Holten A/S Denmark, Type SBD-50 cold), operated at 75 rpm, was used to study the adsorption kinetic and thermodynamic of lac dye onto silk.

(d) Freeze dyer (Heto FD3 model S/N 492497-B, Cat No. 837107, Denmark) was used to dry the crude extract.

3.2.3 Experimental methods

3.2.3.1 Silk yarn preparation

The silk yarn used was purchased from villagers living in Nakhon Ratchasima, Thailand. To remove the sericin gum, the silk yarn (1 kg) was added to boiling water (5 L) which had been added soap flakes (*ca* 100 g), sodium silicate (10 g), sodium carbonate (50 g) and 40% hydrogen peroxide (100 mL). The mixture was then boiled for 2 hours. The silk was then removed, washed with water, squeezed to remove excess liquor and air dried. Finally, it was treated with 1M HCl (*ca* 3 L) at room temperature for 30 minutes and then removed and washed with deionized water until the rinsed water was neutral. The silk yarn was then dried at room temperature.

3.2.3.2 Preparation of lac dye

Stick lac (500.48 g) from the Rain tree, *Samanea saman* (Jacq.) Merr. (*Pithecolobium saman*, Mimosaceae), in northeast Thailand (Nakhon Ratchasima) was finely powdered (18 mesh) in a grinding mill. The powdered material was extracted with deionized water (1.5 L) at 60 ^{o}C for 1 hour. The aqueous solution was filtered and the filtrate concentrated under reduced pressure (rotary evaporator) to give a crude lac dye extract (38.33 g), which was then used without further purification.

Erythrolaccin-free lac dye was also prepared. Stick lac (300 g) was extracted with ethanol (900 mL) for 15 minutes at room temperature and then filtered to remove the erythrolaccin solution. The stick lac remaining was then treated with deionized

water (3.0 L) for 24 hours at room temperature with stirring. The resultant aqueous dye solution was filtered and then the filtrate was concentrated under reduced pressure to obtain the erythrolaccin-free lac dye (9.9628 g). It was used without further purification.

3.2.3.3 Batch kinetic experiments

Lac dye prepared in the section 3.2.3.2 was dissolved in deionized water to the required concentrations. The pH of the dye solutions was adjusted to 3.0 with glacial acetic acid. The dye solution (50 mL) in each conical flask (125 mL) was shaken in a thermostatted shaker bath operated at 75 rpm. After 30 minutes, the silk yarn (0.50 g), which had been pre-warmed in the thermostatted bath for 30 minutes, was immersed in the dye solution. The silk samples were then rapidly withdrawn after different immersion times. Dye concentrations were determined at time zero and at subsequent times using a calibration curve based on absorbance at λ_{max} 487 nm (Cary 1E UV-Visible spectrophotometer) versus dye concentration in standard lac dye solutions. The amount of dye adsorbed per gram of silk (q_t) (mg/g silk) at any time was calculated by a mass-balance relationship (equation (3.20)) as follows:

$$q_t = (C_o - C_t)\frac{V}{W} \qquad (3.20)$$

where C_o and C_t are the initial and dye concentrations (mg/L) after dyeing time t respectively. V is the volume of dye solution (mL) and W is the weight of silk yarn (g) used. Kinetic experiments of lac dyeing onto silk were repeated three times.

3.2.3.4 Silk pretreatment with chitosan

Chitosan (medium molecular weight, viscosity 200.000 cps, CAS 9012-76-4, FW 161) was purchased from the Aldrich Chemical Company. A 1% (w/v) stock solution of chitosan was prepared by dissolving the required amount of chitosan in a 1% (v/v) aqueous acetic acid solution. The silk yarn (50 g), prepared as noted in section 3.2.3.1, was then immersed directly in 0.3% and 0.6% (v/v) aqueous solutions of chitosan (2 L) (prepared from the stock solution) at room temperature for 1 hour. After this time the yarn was removed and dried at 100 ^{o}C for 30 min and cured at 160 ^{o}C for 10 min. The silk yarn, after pre-treatment with chitosan, was rinsed with water at 40 ^{o}C and allowed to dry in the open air in the laboratory.

3.2.3.5 Batch equilibrium experiments

Different lac dye concentrations were freshly prepared in deionized water. The pH of the dye solution was adjusted to 3.0 with glacial acetic acid. The experiments were carried out by shaking silk yarn (0.5 g) with different concentrations of dye solution (50 mL) in a conical flask at 30 and 60 ^{o}C in a thermostatted shaker bath operated at 75 rpm. The amount of dye in the solution was monitored by UV-Visible absorption spectroscopy (Cary 1E UV-Visible spectrophotometer) until the absorbance values at λ_{max} 487 nm remained constant. The initial and equilibrium dye concentrations were determined using a calibration curve based on absorbance at λ_{max} 487 nm versus dye concentration in standard lac dye solutions. Equation (3.21) was used to calculate the amount of dye adsorbed at equilibrium (q_e) (mg/g silk).

$$q_e = (C_O - C_e)\frac{V}{W} \qquad (3.21)$$

In equation (3.21) C_o and C_e are the initial and equilibrium dye solution concentrations (mg/L) respectively, and V is the volume of the dye solution (mL) and W is the weight of silk yarn (g) used. Equilibrium experiments for the adsorption of lac dyeing onto silk were repeated three times.

3.2.3.6 The effect of chitosan on the adsorption and desorption of lac dyeing onto silk

The silk yarn with and without pretreated with chitosan were dyed with an initial dye concentration of 547 mg/L in a thermostatted shaker bath operated at 75 rpm. The dyeing conditions were at a pH 3.0, MLR of 1:100, and 30 ^{o}C. The absorbance of dye solution was monitored until the absorbance values at λ_{max} 487 nm remained constant. The silk yarn was taken out and then dried at room temperature. To study the desorption of lac dye from silk samples at 30 ^{o}C, deionized water (50 mL) in each conical flask (125 mL) was shaken in a thermostatted shaker bath operated at 75 rpm. After 30 minutes, the dried silk sample (0.50 g), which had been pre-warmed in the thermostatted bath at 30 ^{o}C for 30 minutes, was immersed in deionized water. The samples were then rapidly withdrawn after different immersion times. The desorbed dye concentrations (q_{de}) were determined using a calibration curve based on absorbance at λ_{max} 487 nm (Cary 1E UV-Visible spectrophotometer) versus dye concentration in standard lac dye solutions. The amount of dye adsorbed on silk after desorption was calculated by subtraction.

3.3 Results and discussion

3.3.1 Optimal conditions for lac dyeing of silk

In order to investigate the adsorption of lac dye on silk, the experiment parameters including pH, material to liquor ratio (MLR), contact time, initial dye concentration and temperature were determined to find the optimal conditions for adsorption.

3.3.1.1 The effect of pH on the adsorption of lac dye onto silk

Lac dye, is composed mainly of two major anthraquinone-based components: laccaic acids A and B (Burwood *et al*., 1967; Pandhare *et al*.,1969; Oka *et al*., 1998) and the minor components, laccaic acids C, D and E (Rama *et al*., 1968; Mehandale *et al*., 1968) and is classified an acid dye (Saxena *et al*., 1997). The pH of the dye solution is one of the most important parameters controlling the adsorption capacity of dye onto silk (Carr, 1995; Christie *et al*., 2000). The adsorption data of lac dyeing onto silk at different pH values are listed in Table 3.1. The effect of pH on the adsorption of lac dye onto silk at 30 ^{o}C with the initial dye concentration of 548 mg/L and the MLR of 1:100 is shown in Figure 3.3. It indicated that the adsorption capacity increased with decreasing pH over the pH range 4.5-3.5, and remained constant in the pH range 3.5-3.0, but dropped gradually at pH values lower than 3.0. The highest adsorption capacity was observed to be in the pH range of 3.5-3.0. This is due mainly to an increase in the protonation of the amino ($-NH_2$) groups of amino acids at the end of fibroin chain, while the carboxyl groups in the side chains are essentially unionized

Table 3.1 The amount of dye adsorbed per gram of silk at different pH; dyeing at MLR 1:100, an initial dye concentration of 548 mg/L, 30 ^{o}C and 1 hour of contact time

pH of dye solution	Weight silk (g)	A_o	$A_{t = 1\ hr}$	q_e (mg/g silk)
5.49	0.5137	2.2724	1.5417	17.1
4.46	0.5108	2.3530	1.7356	14.5
4.04	0.5104	2.3650	1.5771	18.6
3.53	0.5120	2.3442	0.3020	48.1
3.08	0.5115	2.3242	0.2695	48.4
2.74	0.5117	2.3378	0.4099	45.4

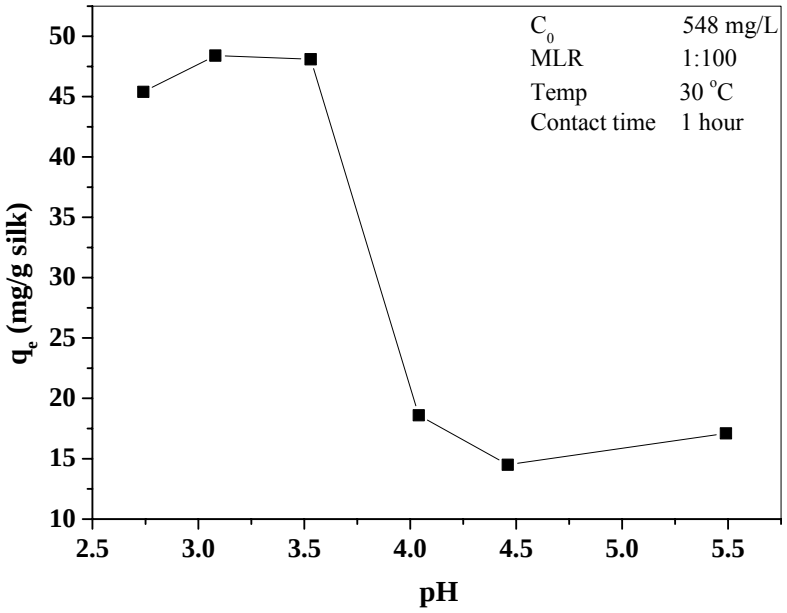


Figure 3.3 The effect of pH on the adsorption of lac dye onto silk at 30 ^{o}C and 1 hour of contact time.

at lower pH. From a model of the electrostatic map of laccaic acid A (Spartan Program; AM1; Wavefunction Inc.; ′02 Linux/Unix) as shown in Figure 3.4, it was found that it has higher negative potential at the 9-quinone carbonyl oxygen group compared with the potential at the other quinone group because of the delocalization of the lone pairs of electrons from the phenolic groups at positions 3 and 6. Therefore,

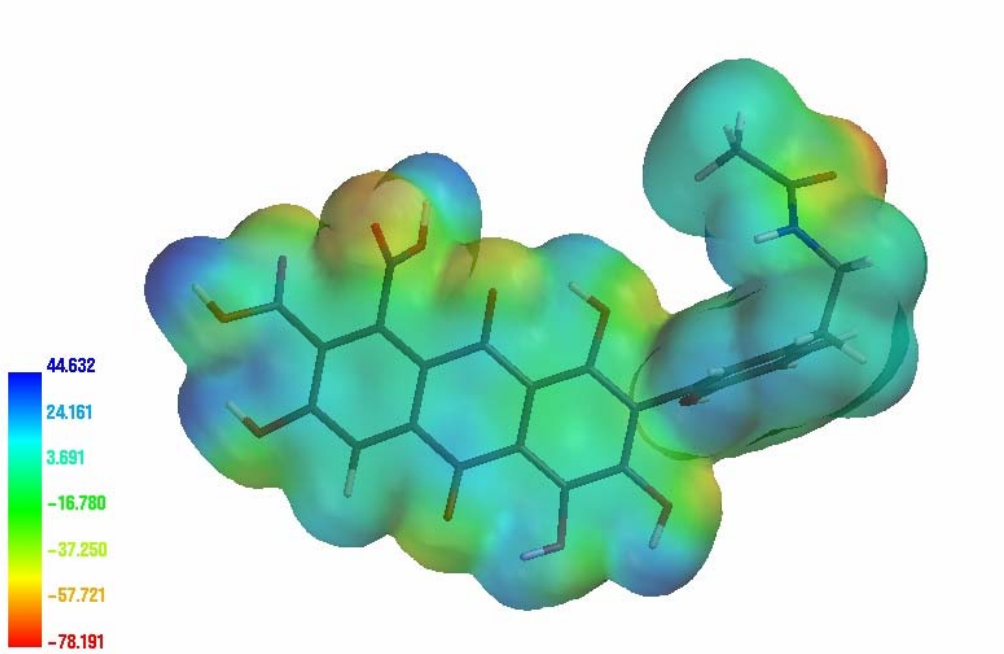


Figure 3.4 Electrostatic map of laccaic acid A (Spartan Program; AM1; Wavefunction Inc.; ′02 Linux /Unix).

the positive charge on silk most probably attracts laccaic acids by electrostatic ion – dipole forces. In addition, laccaic acids can form hydrogen bonds with silk because these structures contain hydroxyl groups. Therefore, it was concluded that lac dye onto silk would be favourable under acidic conditions. The pH of the dye solution in all experiments on the adsorption kinetics was fixed at 3.0. From Table 3.2, the

adsorption of lac dye onto silk at pH 3.0 and 4.0 could be described by the pseudo second-order kinetic model. In addition, the initial dye adsorption rate (h_i) at pH 3.0 was higher than that at pH 4.0, consistent with the dyeing process being favoured under acidic conditions.

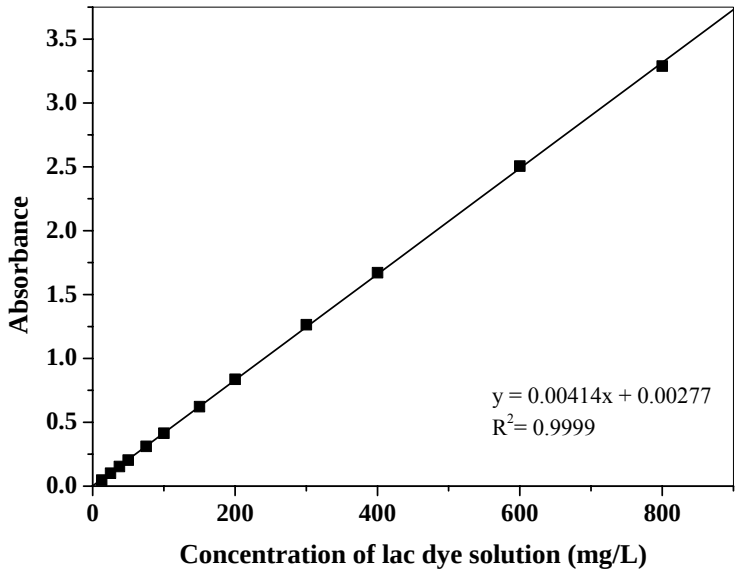


Figure 3.5 Calibration curve of Thai lac dye solution.

Table 3.2 Comparison of the pseudo first- and second-order adsorption rate constants and the calculated and experimental q_e values for different pH, initial dye concentrations and temperatures

Parameters	$q_{e,exp}$	Pseudo first-order model			Pseudo second-order model			
	(mg/g silk)	k_1 (min^{-1})	$q_{e,cal}$ (mg/g silk)	R^2	k_2 (g silk/mg min)	$q_{e,cal}$ (mg/g silk)	h_i (mg/g silk min)	R^2
pH: initial dye concentration (C_o) 534±20 mg/L, contact time 60 min, MLR 1:100, temperature 30 ^oC								
3.0	48.8	8.54×10^{-2}	37.7	0.9938	4.55×10^{-3}	50.1	11.4	0.9977
4.0	12.4	4.51×10^{-2}	10.3	0.9743	1.29×10^{-2}	10.8	1.5	0.9921
Initial dye concentration; C_o (mg/L): contact time 60 min, MLR 1:100, temperature 30 ^oC (pH 3.0)								
127	11.1	8.86×10^{-2}	8.0	0.9883	2.40×10^{-2}	11.2	3.0	0.9944
269	23.0	1.22×10^{-1}	16.3	0.9672	1.26×10^{-2}	27.1	9.3	0.9988
534	48.8	8.54×10^{-2}	37.7	0.9938	4.55×10^{-3}	50.1	11.4	0.9977
Temperature (^oC): initial dye concentration (C_o) 534±20 mg/L, contact time 60 min, MLR 1:100 (pH 3.0)								
30	48.8	8.54×10^{-2}	37.7	0.9938	4.55×10^{-3}	50.1	11.4	0.9977
40	47.0	1.14×10^{-1}	28.8	0.9819	8.67×10^{-3}	48.6	20.5	0.9990
60	41.9	1.17×10^{-1}	15.0	0.9423	2.49×10^{-2}	42.1	43.7	0.9997

3.3.1.2 The effect of material to liquor ratio (MLR) on the adsorption of lac dye onto silk

The aim of dyeing is to transfer the dye molecules from the dye liquor to the fiber in a uniform and efficient manner. The rate of dye uptake by the fiber is significantly increased by the movement of the dye liquor relative to the fiber (Christie *et al*., 2000). The effect of material to liquor ratio (MLR) on the adsorption of lac dye onto silk is shown in Figure 3.6. It was found that an increase in volume of dye solution resulted in an increase of the dye adsorbed onto silk. It indicated that silk yarn is loosely packed in the higher volume of dye solution and the dye solution readily moves past any surface transferring dye molecules to the silk surface in the process. On reaching the silk surface the dye molecules will be adsorbed into the surface and then diffuse into the interior of the silk yarn. Since an MLR of 1:100 or 1:150 showed only a small difference in the amount of the dye adsorbed onto the silk, the MLR of 1:100 ratio was used for all the kinetic experiments.

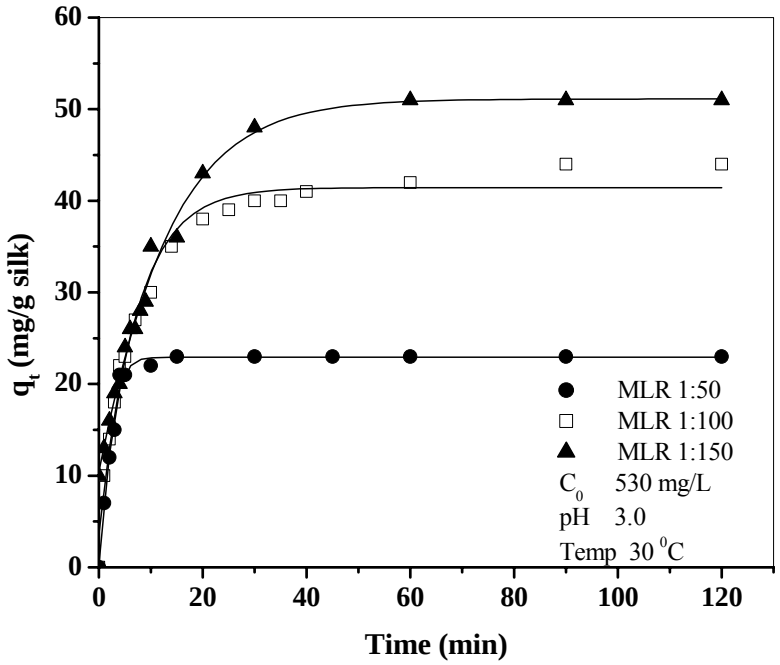


Figure 3.6 The effect of material to liquor ratio on the adsorption of lac dye onto silk.

3.3.1.3 The effect of contact time and initial dye concentration on the adsorption of lac dye onto silk

The adsorption of lac dye at different initial dye concentrations onto silk was investigated as a function of contact time in order to determine the equilibrium time for maximum adsorption. The adsorption data under different initial dye concentrations for lac dyeing onto silk are listed in Tables 3.3-3.5. A plot of the amount of dye adsorbed per gram silk (q_t) (mg/g silk) at any time versus contact time (t) is shown in Figure 3.7. It was found that the adsorption capacity is concentration dependent and increased with initial concentration of the lac dye. An increase in the initial dye concentration led to an increase in the amount of dye adsorbed onto silk. This may be a result of an increase in the driving force of the concentration gradient

with the increase in the initial dye concentration (Chiou and Li, 2002). This indicated that the initial dye concentration plays an important role in the adsorption capacity of lac dye onto silk. The results of rate constant studies for different initial dye concentrations by using the pseudo-first order and second-order kinetic models are listed in Table 3.2. The second-order kinetic model well described the adsorption of lac dye onto silk with a high correlation coefficient ($R^2 > 0.99$) as shown in Figure 3.8; calculated equilibrium adsorption capacity ($q_{e,cal}$) was only slightly different from the experimental data. This suggested that the overall rate of the lac dye adsorption onto silk is controlled by the chemisorption. From Table 3.2, the rate constant (k_2) for the pseudo second-order kinetic model decreased with increasing initial dye concentration whereas the initial dye adsorption rate (h_i) increased with an increasing initial dye concentration. The equilibrium time is the time taken for the maximum adsorption of dye onto the silk surface, above which the adsorption remains constant. The equilibrium time was found to be about 30 minutes for 534, 269 and 127 mg/L dye concentration at pH 3.0 and 30 ^{o}C. The adsorption was very fast at the initial stages of contact time and gradually decreased with time until it remained constant. An initial dye concentration of 530±20 mg/L was used throughout this study. Using different initial concentrations in the temperature range 30-60 ^{o}C showed similar trends.

Table 3.3 The adsorption data with an initial dye concentration of 127 mg/L, MLR 1:100, pH 3.0 and 30 ^{o}C

Time (min)	**Weight silk (g)**	**A_o**	**A_t**	**q_t (mg/ g silk)**	**$q_e - q_t$ (mg/ g silk)**	**$\ln(q_e - q_t)$**	**t/q_t (g silk min/mg)**
0	-	0.5306	-	0.0	-	-	-
1	0.5010	0.5306	0.3942	3.2	8.6	2.15	0.310
2	0.5020	0.5306	0.3406	4.5	7.3	1.99	0.444
3	0.5022	0.5306	0.3151	5.1	6.7	1.90	0.586
4	0.5023	0.5306	0.2979	5.5	6.3	1.84	0.724
5	0.5026	0.5306	0.2373	7.0	4.8	1.57	0.716
6	0.5011	0.5306	0.2114	7.6	4.2	1.43	0.787
7	0.5016	0.5306	0.2160	7.5	4.3	1.46	0.932
8	0.5008	0.5306	0.2037	7.8	4.0	1.38	1.023
9	0.5045	0.5306	0.1725	8.5	3.3	1.19	1.058
10	0.5025	0.5306	0.1932	8.0	3.8	1.32	1.243
15	0.5020	0.5306	0.1216	9.8	2.0	0.71	1.535
20	0.5044	0.5306	0.1030	10.2	1.6	0.49	1.966
30	0.5015	0.5306	0.0732	10.9			
60	0.5022	0.5306	0.0472	11.6			
90	0.5004	0.5306	0.0391	11.8			
120	0.5015	0.5306	0.0386	11.8			

Table 3.4 The adsorption data with an initial dye concentration of 269 mg/L, MLR 1:100, pH 3.0 and 30 ^{o}C

Time (min)	**Weight silk (g)**	**A_o**	**A_t**	**q_t (mg/ g silk)**	**$q_e - q_t$ (mg/ g silk)**	**$\ln(q_e - q_t)$**	**t/q_t (g silk min/mg)**
0	-	1.1175	-	0.0	-	-	-
1	0.5046	1.1175	0.7996	7.5	18.0	2.89	0.133
2	0.5031	1.1175	0.6634	10.8	14.7	2.69	0.185
3	0.5053	1.1175	0.5505	13.5	12.0	2.49	0.222
4	0.5043	1.1175	0.5209	14.2	11.3	2.42	0.281
5	0.5038	1.1175	0.3669	17.9	7.6	2.02	0.279
7	0.5044	1.1175	0.3064	19.4	6.1	1.82	0.362
10	0.5040	1.1175	0.2276	21.3	4.2	1.44	0.470
15	0.5025	1.1175	0.1783	22.5	3.0	1.08	0.665
20	0.5046	1.1175	0.1271	23.6	1.9	0.62	0.846
30	0.5040	1.1175	0.0941	24.5	1.0		
45	0.5039	1.1175	0.0715	25.0	0.5		
60	0.5038	1.1175	0.0674	25.1	0.4		
120	0.5037	1.1175	0.0533	25.5	0.0		
180	0.5064	1.1175	0.0495	25.4	0.1		
240	0.5049	1.1175	0.0478	25.5	0.0		
300	0.5050	1.1175	0.0512	25.4	0.1		
420	0.5051	1.1175	0.0468	25.5	0.0		

Table 3.5 The adsorption data with an initial dye concentration of 534 mg/L, MLR 1:100, pH 3.0 and 30 ^{o}C

Time (min)	**Weight silk (g)**	**A_o**	**A_t**	**q_t (mg/ g silk)**	**$q_e - q_t$ (mg/ g silk)**	**$\ln(q_e - q_t)$**	**t/q_t (g silk min/mg)**
0	-	2.2139	-	0.0	-	-	-
1	0.5060	2.2139	1.7442	11.1	37.9	3.63	0.090
2	0.5076	2.2139	1.5740	15.1	33.9	3.52	0.132
3	0.5097	2.2139	1.3624	20.1	28.9	3.36	0.149
4	0.5054	2.2139	1.2117	23.9	25.1	3.22	0.168
5	0.5058	2.2139	1.1420	25.5	23.5	3.16	0.196
7	0.5035	2.2139	0.9748	29.6	19.4	2.96	0.236
10	0.5063	2.2139	0.8095	33.4	15.6	2.75	0.299
14	0.5058	2.2139	0.6040	38.3	10.7	2.37	0.365
20	0.5044	2.2139	0.4718	41.6	7.4	2.00	0.481
25	0.5056	2.2139	0.4075	43.1	5.9		
30	0.5040	2.2139	0.3742	44.0	5.0		
35	0.5063	2.2139	0.3483	44.4	4.6		
40	0.5059	2.2139	0.2836	46.0	3.0		
60	0.5048	2.2139	0.2422	47.1	1.9		
120	0.5037	2.2139	0.1948	48.3	0.7		
180	0.5035	2.2139	0.1779	48.7	0.3		
240	0.5048	2.2139	0.1667	48.9	0.1		
300	0.5072	2.2139	0.1668	48.6	0.4		
420	0.5055	2.2139	0.1667	48.8	0.2		

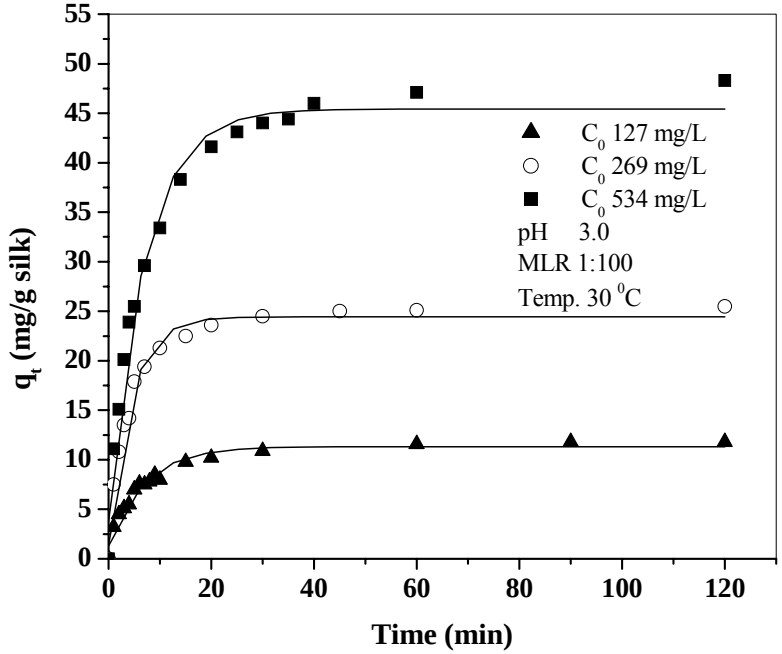


Figure 3.7 The effect of initial dye concentration on the adsorption of lac dye onto silk.

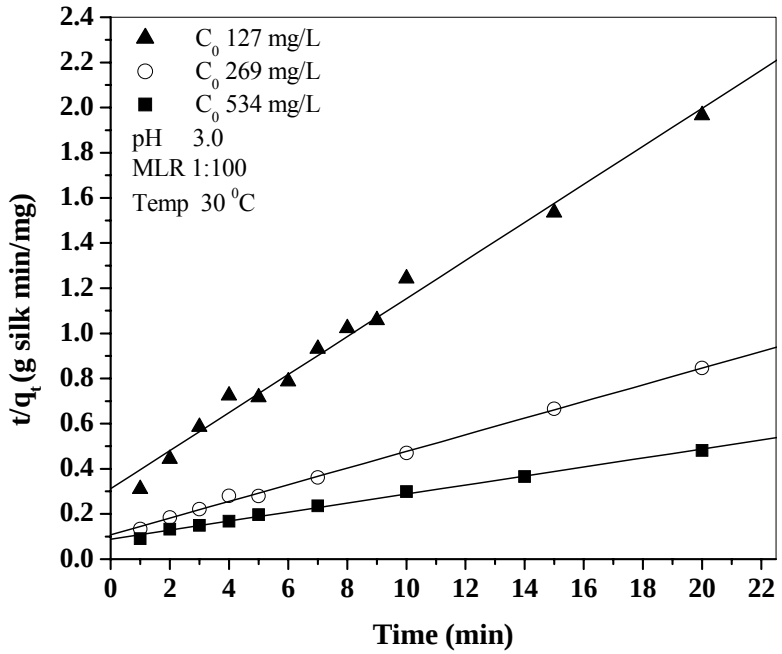


Figure 3.8 The pseudo second-order equation at different initial dye concentrations.

3.3.1.4 The effect of temperature on the adsorption of lac dye onto silk

The adsorption data of lac dyeing on silk at 30, 40 and 60 ^{o}C are given in Tables 3.3, 3.6 and 3.7 respectively.

Table 3.6 The adsorption data with an initial dye concentration of 514 mg/L, MLR 1:100, pH 3.0 and 40 ^{o}C

Time (min)	Weight silk (g)	A_o	A_t	q_t (mg/ g silk)	$q_e - q_t$ (mg/ g silk)	$\ln(q_e - q_t)$	t/q_t (g silk min/mg)
0	-	2.1295	-	0.0	-	-	-
1	0.5048	2.1295	1.4493	16.2	30.8	3.43	0.062
2	0.5018	2.1295	1.1982	22.3	24.7	3.20	0.089
3	0.5037	2.1295	1.1062	24.5	22.5	3.11	0.123
4	0.5030	2.1295	0.8505	30.6	16.4	2.79	0.131
5	0.5010	2.1295	0.7279	33.7	13.3	2.59	0.148
7	0.5053	2.1295	0.5266	38.2	8.8	2.17	0.183
10	0.5029	2.1295	0.4827	39.5	7.5	2.02	0.253
15	0.5012	2.1295	0.4176	41.2	5.8	1.76	0.364
20	0.5027	2.1295	0.3017	43.8	3.2	1.15	0.456
25	0.5033	2.1295	0.2737	44.5	2.5		
30	0.5048	2.1295	0.2582	44.7	2.3		
35	0.5053	2.1295	0.2419	45.0	2.0		
40	0.5060	2.1295	0.2301	45.3	1.7		
60	0.5076	2.1295	0.2186	45.4	1.6		
120	0.5077	2.1295	0.1930	46.0	1.0		
180	0.5083	2.1295	0.1768	46.3	0.7		
240	0.5050	2.1295	0.1777	46.6	0.4		
300	0.5050	2.1295	0.1787	46.6	0.4		
420	0.5000	2.1295	0.1776	47.0			

Table 3.7 The adsorption data with an initial dye concentration of 525 mg/L, MLR 1:100, pH 3.0 and 60 ^{o}C

Time (min)	**Weight silk (g)**	**A_o**	**A_t**	**q_t (mg/ g silk)**	**$q_e - q_t$ (mg/ g silk)**	**$\ln(q_e - q_t)$**	**t/q_t (g silk min/mg)**
0	-	2.1763	-	0.0	-	-	-
1	0.5075	2.1763	1.1512	24.3	17.7	2.87	0.041
2	0.5070	2.1763	1.0663	26.4	15.6	2.75	0.076
3	0.5053	2.1763	0.9000	30.4	11.6	2.45	0.099
4	0.5052	2.1763	0.7641	33.7	8.3	2.12	0.119
5	0.5030	2.1763	0.6633	36.3	5.7	1.75	0.138
7	0.5025	2.1763	0.5870	38.1	3.9	1.35	0.184
10	0.5080	2.1763	0.5354	38.9	3.1	1.12	0.257
15	0.5057	2.1763	0.5222	39.4	2.6	0.94	0.380
20	0.5081	2.1763	0.4875	40.1	1.9	0.65	0.499
25	0.5077	2.1763	0.4684	40.6	1.4		
30	0.5027	2.1763	0.4899	40.4	1.6		
35	0.5030	2.1763	0.4890	40.4	1.6		
40	0.5037	2.1763	0.4789	40.6	1.4		
60	0.5033	2.1763	0.4030	42.5	-0.5		
120	0.5063	2.1763	0.4269	41.7	0.3		
180	0.5055	2.1763	0.4130	42.1	-0.1		
240	0.5066	2.1763	0.4200	41.8	0.2		
300	0.5057	2.1763	0.4232	41.8	0.2		
420	0.5051	2.1763	0.4200	41.9	0.1		

The results of the studies on the influence of temperature on the adsorption of lac dye onto silk are shown in Figure 3.9. It was carried out under the optimal conditions of pH 3.0, MLR 1:100 and an initial dye concentration of 534±20 mg/L. Before and after the equilibrium time, the amount of dye adsorbed per gram of silk (q_t) showed different trends at different temperatures. Before the equilibrium time, the initial dye adsorption rate (h_i) increased with increasing temperature which indicated a kinetically controlled process as shown in Table 3.2. This result may reflect an increase in the mobility of the large dye ions with temperature and thus an increase in the number of molecules interacting with the active sites at the surface. After the equilibrium time, the decrease of the amount of the dye adsorbed per gram of silk with increasing temperature indicated that the adsorption of lac dye onto silk was controlled by an exothermic process. The equilibrium of the lac dyeing process was shifted to the left-hand side. Therefore, the amount of dye adsorbed at high temperature was lower than that at low temperature after the equilibrium time. This behaviour is similar to that observed for the adsorption of reactive dye and anionic dye on cross-linked chitosan beads (Choy *et al*., 1999; Chiou and Li, 2002). Our data showed that the time to reach the adsorption equilibrium decreased with increasing temperature, *i.e.* 60, 25, and 15 min at 30, 40 and 60 ^{o}C respectively. This is due to more rapid diffusion to the silk with higher temperatures. However, the contact time in the adsorption experiments was set at 60 minutes throughout this study.

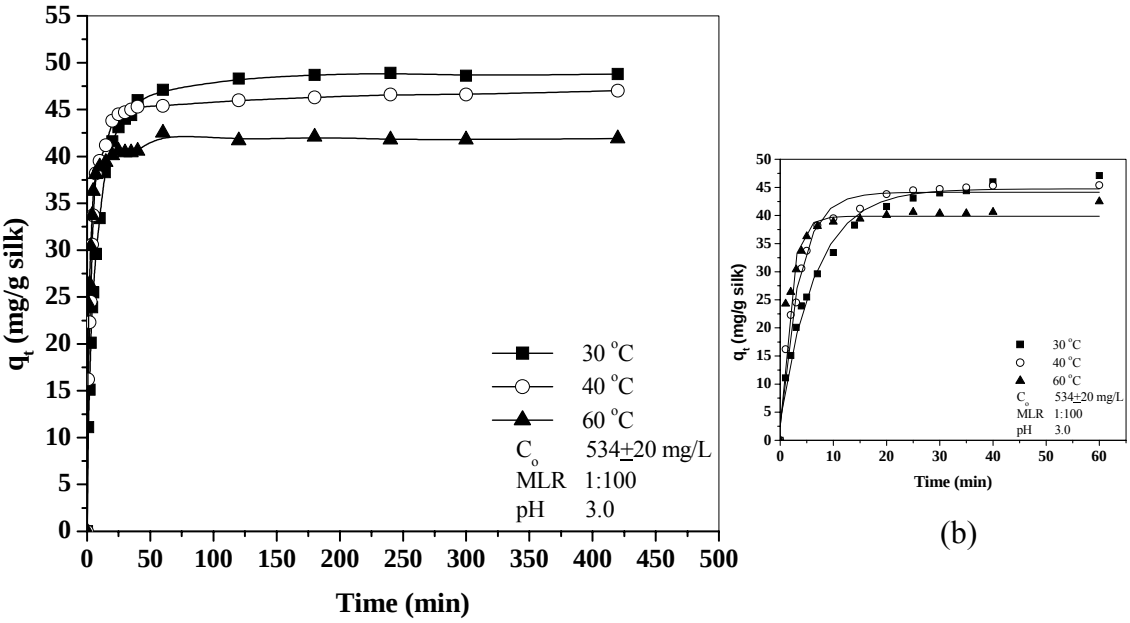


Figure 3.9 The effect of contact time and temperature of lac dye onto silk at the initial dye concentration of 530±20 mg/L, MLR 1:100 and pH 3.0.

(a) The effect of contact time and temperature in the period of 0-420 min.

(b) The effect of contact time and temperature in the period of 0-60 min (expansion of graph 'a').

The optimal conditions obtained from this study at pH 3.0 and MLR 1:100 were subsequently used to study the kinetic and adsorption isotherm of lac dyeing.

3.3.2 Adsorption isotherm

3.3.2.1 The Langmuir isotherm

The experimental data for the adsorption of lac dye on silk were fitted to a linear form of Langmuir isotherm by the equation (3.13). The data for the adsorption of lac dyeing on silk at 30 and 60 ^{o}C are listed in Tables 3.8 and 3.9.

Table 3.8 Data for the adsorption isotherm of lac dyeing onto silk at the initial dye concentration range 120-443 mg/L, MLR 1:100, pH 3.0 and 30 ^{o}C

Initial conc. (mg/L)	**Weight silk (g)**	**A_o**	**$A_{t\ =\ 1\ hr}$**	**q_e (mg/ g silk)**	**C_e (mg/mL)**	**$1/q_e$ (g silk /mg)**	**$1/C_e$ (mL/mg)**
120	0.5036	0.5021	0.1082	9.28	0.0238	0.1077	42.01
149	0.5032	0.6219	0.1295	11.68	0.0290	0.0856	34.48
201	0.5065	0.8357	0.1934	15.21	0.0446	0.0657	22.43
250	0.5056	1.0343	0.2485	18.70	0.0580	0.0535	17.23
298	0.5024	1.2335	0.2821	22.84	0.0662	0.0438	15.10
348	0.5060	1.4390	0.3738	25.42	0.0886	0.0393	11.29
443	0.5015	1.8265	0.4984	32.04	0.1190	0.0312	8.41

Table 3.9 Data for the adsorption isotherm of lac dyeing onto silk at the initial dye concentration range 120-443 mg/L, MLR 1:100, pH 3.0 and 60 ^{o}C

Initial conc. (mg/L)	**Weight silk (g)**	**A_o**	**A_t**	**q_e (mg/ g silk)**	**C_e (mg/mL)**	**$1/q_e$ (g silk /mg)**	**$1/C_e$ (mL/mg)**
120	0.5053	0.5021	0.2241	6.45	0.0521	0.1550	19.20
149	0.5025	0.6219	0.2726	8.22	0.0639	0.1217	15.65
201	0.5023	0.8357	0.3861	10.66	0.0916	0.0938	10.92
250	0.5045	1.0343	0.5021	12.61	0.1199	0.0793	8.34
298	0.5048	1.2335	0.5665	15.86	0.1356	0.0631	7.38
348	0.5038	1.4390	0.7173	17.21	0.1724	0.0581	5.80
443	0.5032	1.8265	0.9183	21.75	0.2214	0.0460	4.52

The linear plot of $1/q_e$ versus $1/C_e$ is obtained from this model as shown in Figure 3.10. The values of Q and b were calculated from the intercepts and slopes of different straight lines representing the different temperatures; Table 3.10 lists the calculated results. The fit is good for the adsorption data of lac dye onto silk at 30 and 60 ^{o}C (correlation coefficient, $R^2 > 0.99$). It was found that the adsorption of lac dye at higher temperature decreased with increasing temperature indicating that the process is exothermic. As expected, the Q values decreased with increasing temperature. The b values indicated that the silk yarn has a maximum affinity for lac dye at lower temperature.

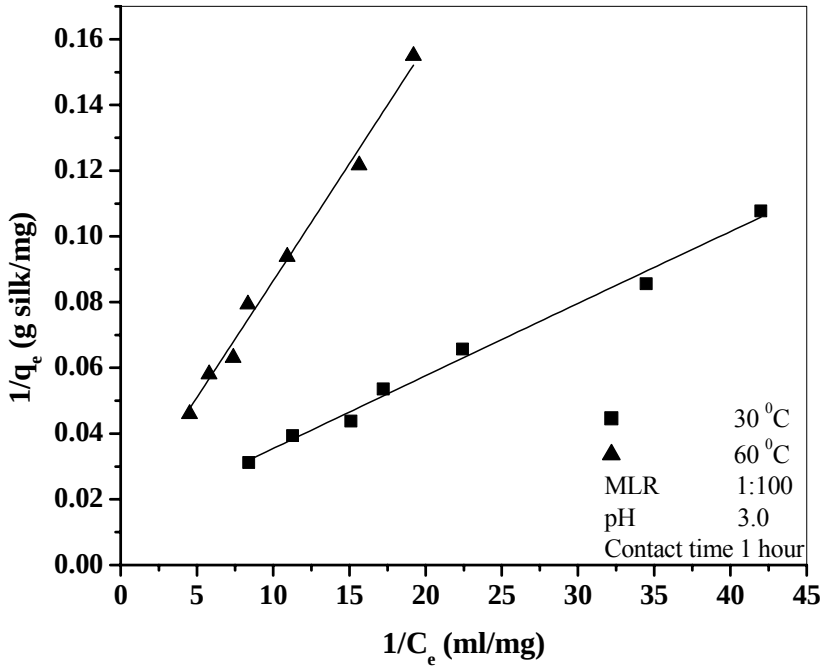


Figure 3.10 Langmuir adsorption isotherm of lac dye onto silk at 30 and 60 °C.

Table 3.10 Langmuir and Freundlich isotherm constants for the adsorption of lac dye onto silk at different temperatures

Temperature	**Langmuir**			**Freundlich**		
(°C)	***Q***	***b***	***R²***	***Q_f***	***n***	***R²***
	(mg/g cotton)	**(mL/mg)**		**(mg/g cotton)**		
30	74.0	6.14	0.9956	163.5	1.32	0.9947
60	67.9	2.06	0.9955	75.0	1.22	0.9942

The equilibrium parameter values, R_L, for the adsorption of lac dyeing on silk were calculated by using equation (3.14). It was found that the values of R_L (Table 3.11) were observed to be in the range of 0-1, indicating that the adsorption of lac dye onto silk was favourable for this study.

Table 3.11 Data for Langmuir isotherm for lac dyeing of silk

Temperature (^oC)	***b*** **(L/mg)**	**Initial dye concentration C_o (mg/L)**	R_L
30	6.14×10^{-3}	443	0.2689
		348	0.3186
		298	0.3532
		250	0.3948
		201	0.4473
		149	0.5221
		120	0.5760
60	2.06×10^{-3}	443	0.5229
		348	0.5822
		298	0.6194
		250	0.6604
		121	0.7069
		149	0.7650
		120	0.8020

3.3.2.2 The Freundlich isotherm

The Freundlich equation was also applied for the adsorption of lac dyeing by the equation (3.16). The linear plot of ln q_e versus ln C_e is shown in Figure 3.11. Therefore, Q_f and 1/n can be determined from intercept and slope of this plot as listed in Table 3.10. The magnitude of the exponent 1/n gives an indication of the favourability of adsorption. Values, of $n > 1$ obtained represent favourable adsorption conditions (Chiou and Li, 2002). The Q_f values decreased with increasing temperature which again supported an exothermic process. Therefore, the Freundlich equation can be applied to fit the experimental data as well as the Langmuir equation because it gave a high correlation coefficient ($R^2 > 0.99$).

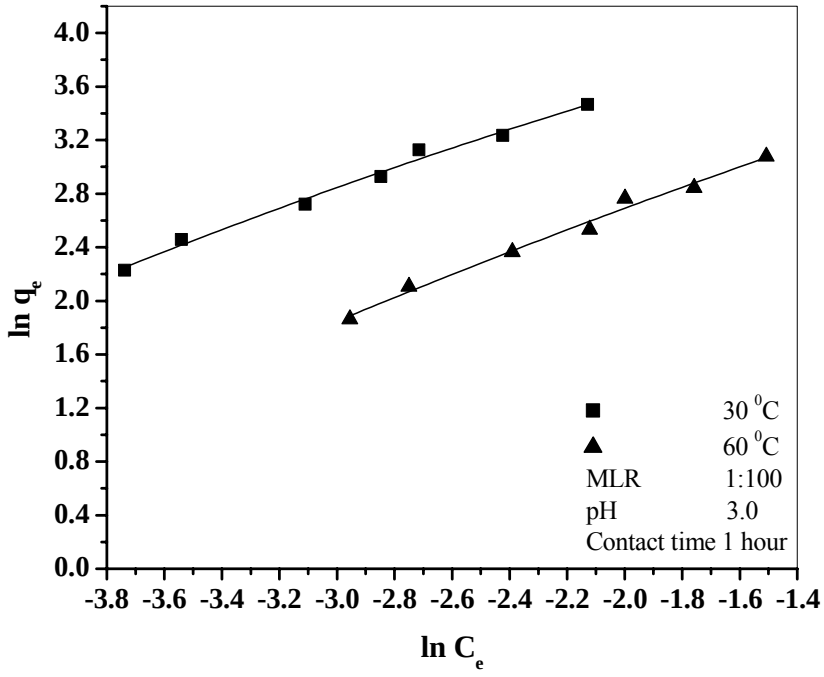


Figure 3.11 Freundlich adsorption isotherm of lac dye onto silk at 30 and 60 ^{o}C.

3.3.3 Kinetics of adsorption

Kinetic data were treated with Lagergren's pseudo first order equation (3.2). A straight line of ln (q_e - q_t) versus t suggests the applicability of this kinetic model to fit the experimental data. The first-order rate constant k_1 and equilibrium adsorption capacity $q_{e,cal}$ at three different temperatures were calculated from the slope and intercept obtained form the plot of ln (q_e - q_t) versus t (Figure 3.12 and Table 3.2).

The correlation coefficients for the pseudo first-order kinetic model are in the range of 0.96-0.99 (Table 3.2). However, a large equilibrium adsorption density (q_e) difference between the experiment and calculation was observed, indicating a poor pseudo first-order fit to the experimental data.

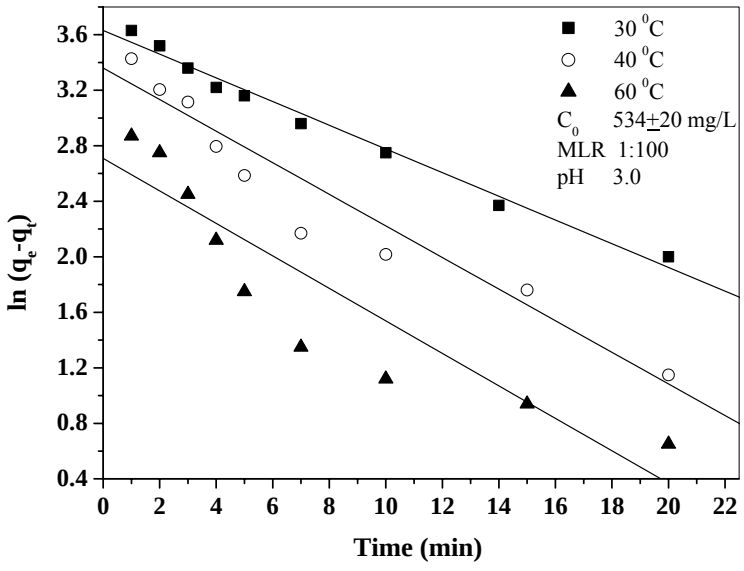


Figure 3.12 Plot of the pseudo first-order equation at different temperatures.

The pseudo second-order kinetic model by the equation (3.5) was also used to fit the experimental data. The slope and intercept of (t/q_t) versus t were used to calculate the pseudo second-order rate constant k_2 and q_e. From Table 3.2, lac dyeing onto silk is considered to be pseudo second-order with high correlation coefficients above 0.99 as shown in Figure 3.13. In addition, the q_e which is the adsorption capacity, agreed very well with both experimental and calculation as listed in Table 3.2. These suggested that the pseudo second-order adsorption mechanism is predominant and that the overall rate of the lac dye adsorption process is most likely to be controlled by the chemisorption process (Chiou and Li, 2002; Chiou *et al*., 2004).

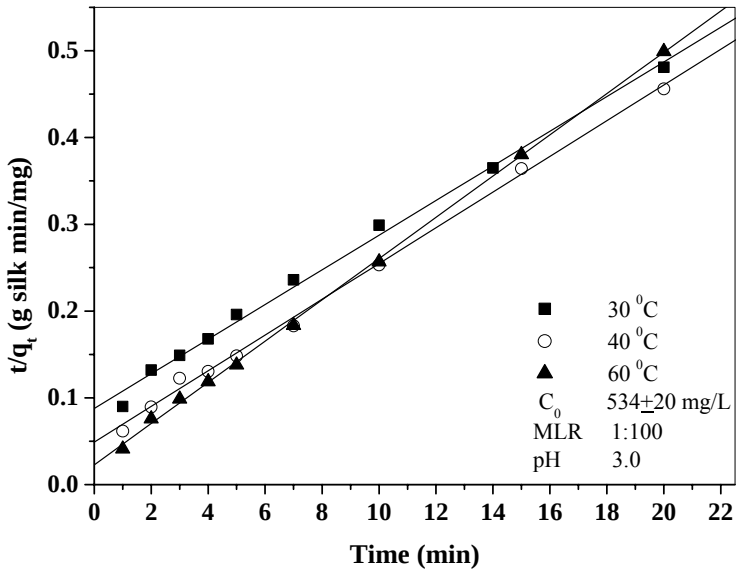


Figure 3.13 Plot of the pseudo second-order equation at different temperatures.

3.3.4 Activation parameters

The rate constant k_2 at different temperatures listed in Table 3.2 were then applied to estimate the activation energy of the adsorption of lac dye onto silk by the Arrhenius equation (Dogan and Alkan, 2003). The slope of the plot of ln k_2 versus 1/T (Figure 3.14) was used to evaluate E_a as listed in Table 3.12.

Table 3.12 Activation parameters for the adsorption of lac dye onto silk

Temp (^oC)	k_2 (g silk/mg second)	E_a (kJ/mol)	R^2	$\Delta H^\#$ (kJ/mol)	$\Delta S^\#$ (J/mol K)	$\Delta G^\#$ (kJ/mol)	R^2
30	7.58 x 10^{-5}					87.8	
40	1.45 x 10^{-4}	47.0	0.9985	44.5	-143.0	89.3	0.9982
60	4.15 x 10^{-4}					92.1	

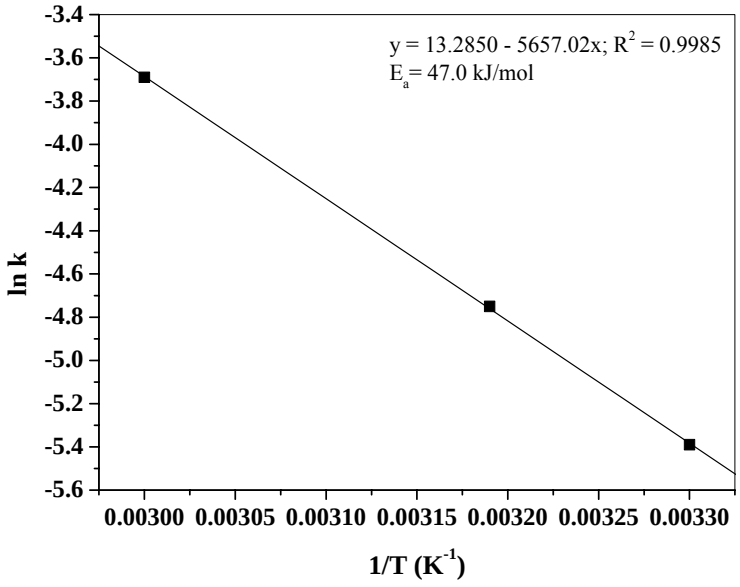


Figure 3.14 Arrhenius plots for the adsorption of lac dye onto silk.

The observed activation energy (E_a) and enthalpy of activation ($\Delta H^{\#}$) for lac dye onto silk yarn shown in Table 3.13 agreed well with the one calculated from the activated complex theory of reaction in solution, $E_a = \Delta H^{\#} + RT$. However, the observed activation energy (E_a) seems to be small for adsorption of lac dye onto silk yarn.

From the Eyring equation, the enthalpy ($\Delta H^{\#}$) and entropy ($\Delta S^{\#}$) of activation were calculated from the slope and intercept of a plot of ln (k/T) versus $1/T$ (Figure 3.15) as listed in Table 3.13. The value of $\Delta G^{\#}$ was calculated at 303, 313 and 333 K by using equation (3.10) and these values are listed in Table 3.13, while the negative entropy value ($\Delta S^{\#}$) reflects the interaction between lac dye and silk yarn.

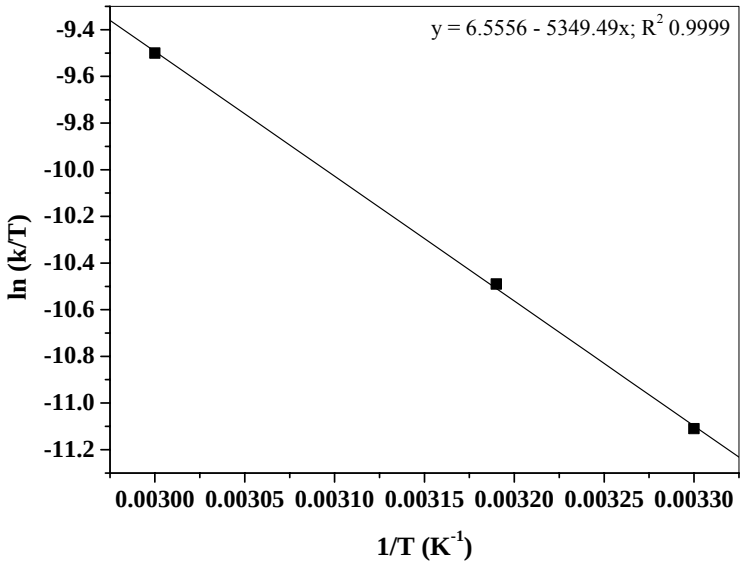


Figure 3.15 Plot of ln (k/T) against $1/T$ for the adsorption of lac dye onto silk.

In order to support the exothermic behaviour of lac dye onto silk, after reaching equilibrium, the thermodynamic parameters, ΔG^o, ΔH^o and ΔS^o of lac dye adsorption, were calculated by using the equations 3.16-3.18 and the results are shown in Table 3.13.

Table 3.13 Thermodynamic parameters for the adsorption of lac dyeing onto silk at different temperatures, MLR 1:100, initial dye concentration 530±20 mg/L and pH 3.0

Temperature (^{o}C)	K_c	ΔG^o (kJ/mol)	ΔH^o (kJ/mol)	ΔS^o (J/mol K)	R^2
30	1214.6	-17.9			
40	1088.1	-18.2	-31.4	-81.9	0.9712
60	413.0	-16.7			

The negative value of ΔG^o and ΔH^o indicated that lac dye adsorption is spontaneous and an exothermic process. The obtained ΔS^o is a negative value.

3.3.5 Comparison of activation parameters between crude lac dye with and without erythrolaccin removal

Crude lac dyes are composed mainly of laccaic acids and a yellow pigment called erythrolaccin (Bhide *et al*., 1965; Pandhare, 1996). They are usually used in dyeing by villagers in northeast Thailand without removing the erythrolaccin (Moeyes, 1993). However, in order to study any effect of erythrolaccin on dye uptake,

kinetic data with and without erythrolaccin being present was obtained. The results are shown in Table 3.14

The activation parameters with and without erythrolaccin in the lac dye are similar indicating only a slight effect of erythrolaccin on lac dyeing onto silk. In particular, $\Delta H^{\#}$ was slightly higher in the latter case while $\Delta S^{\#}$ was smaller.

Table 3.14 Activation parameters for the adsorption of crude lac dye with and without erythrolaccin removal

Crude extract	Temp (^{o}C)	k_2 (g silk/mg second)	E_a (kJ/mol)	R^2	$\Delta H^{\#}$ (kJ/mol)	$\Delta S^{\#}$ (J/mol K)	$\Delta G^{\#}$ (kJ/mol)	R^2
Erythrolaccin present	30	7.58×10^{-5}	47.0	0.9985	44.5	-143.0	87.8	0.9982
	40	1.45×10^{-4}					89.3	
	60	4.15×10^{-4}					92.1	
Erythrolaccin removed	30	6.97×10^{-5}	50.8	0.9969	48.3	-164.9	98.2	0.9966
	40	1.47×10^{-4}					99.9	
	60	4.48×10^{-4}					103.1	

3.3.6 The effect of chitosan on the adsorption and desorption of lac dyeing onto silk

Chitosan has been applied to coat the surface of natural silk fibres (Kweon, Ha, Um and Park, 2001; Siraleartmukul and Chandrkrachang, 2000) in the dyeing process. For instance, the modification of silk fabric with chitosan has been carried out by Waly, Bendak and Abo El Ola (2001). It was found that the

pretreatment of silk with chitosan increased the dye uptake and improved the light yellowing resistance compared to the untreated silk. Also, an increase in dyeability of tussah silk fabric after pretreatment with chitosan under acidic conditions was observed compared to the untreated fabric (Kako, 2000). For this reason, chitosan has been used to treat silk yarn in order to enhance the dye uptake of lac dye in this research. The silk yarn was treated with 0.3% and 0.6% (v/v) aqueous solutions of chitosan. It was found that the pretreated silk with a 0.6% (v/v) aqueous solution of chitosan provided an unsmooth coating which resulted in an uneven distribution of dye adsorbed on silk yarn. Therefore, the silk yarn treated with a 0.3% (v/v) aqueous solution of chitosan was chosen in order to study the dyeability compared to untreated silk at an initial dye concentration of 547 mg/L, MLR 1:100, pH 3.0, and 30 ^{o}C. The amount of the dye adsorbed in this study is listed in Tables 3.15 and 3.16 respectively.

Table 3.15 The adsorption-desorption data of lac dyeing onto untreated silk with chitosan at an initial dye concentration of 547 mg/L, MLR 1:100, pH 3.0 and 30 ^{o}C

Time (min)	**Weight silk (g)**	**A_o**	**A_t**	**q_t (mg/ g silk)**	**Time for desorption (min)**	**q_{de} (mg/ g silk)**	**q_{ad}- q_{de} (mg/ g silk)**
0	-	2.2691	-	0.00	181	5.4	35.3
1	0.5003	2.2691	1.7996	10.9	182	8.2	32.5
2	0.5049	2.2691	1.6018	15.5	185	12.9	27.8
3	0.5017	2.2691	1.4880	18.4	190	16.4	24.3
4	0.5025	2.2691	1.3589	21.5	200	20.7	20.0
5	0.5022	2.2691	1.2537	24.0	210	22.3	18.4
6	0.5053	2.2691	1.1780	25.7	240	22.9	17.8
8	0.5000	2.2691	1.0559	28.9	300	26.4	14.3
10	0.5041	2.2691	0.8885	32.7	360	23.6	17.1
20	0.5012	2.2691	0.7264	36.7	420	23.9	16.8
30	0.5043	2.2691	0.6771	37.7			
60	0.5130	2.2691	0.6153	38.5			
90	0.5060	2.2691	0.6185	39.0			
120	0.5014	2.2691	0.6060	39.6			
180	0.5064	2.2691	0.5441	40.7 (q_e)			

Table 3.16 The adsorption-desorption data of lac dyeing onto pretreated silk with chitosan at an initial dye concentration of 547 mg/L, MLR 1:100, pH 3.0 and 30 ^{o}C

Time (min)	**Weight silk (g)**	**A_o**	**A_t**	**q_t (mg/ g silk)**	**Time for desorption (min)**	**q_{de} (mg/ g silk)**	**q_e- q_{de} (mg/ g silk)**
0	-	2.2691	-	0.00	181	7.2	41.1
1	0.5052	2.2691	1.6336	15.1	182	8.2	40.1
2	0.5027	2.2691	1.3708	21.5	185	10.6	37.7
3	0.5017	2.2691	1.2211	25.2	190	13.8	34.5
4	0.5027	2.2691	1.0938	28.2	200	15.5	32.8
5	0.5010	2.2691	1.0784	28.6	210	17.3	31.0
6	0.5044	2.2691	0.9657	31.1	240	18.0	30.3
8	0.5009	2.2691	0.8286	34.7	300	17.6	30.7
10	0.5038	2.2691	0.6797	38.0	360	16.6	31.7
20	0.5076	2.2691	0.4838	42.4	420	17.5	30.8
30	0.5022	2.2691	0.392	45.1			
60	0.5010	2.2691	0.3153	47.0			
90	0.5004	2.2691	0.2884	47.7			
120	0.502	2.2691	0.2704	48.0			
180	0.5007	2.2691	0.2639	48.3 (q_e)			

Under the same conditions, chitosan increased the adsorption ability of lac dye onto silk yarn compared to the untreated silk as shown in Figure 3.16. This can be explained by the fact that the –NH– and C=O groups of silk fibroin can form hydrogen bonds with the amino groups of chitosan. This is in agreement with the experimental results of Kweon *et al*. (2001) and Park, Oh, Yoo and Shin (2000). They reported that in the FT-IR spectra of chitosan and silk fibroin/chitosan fibre,

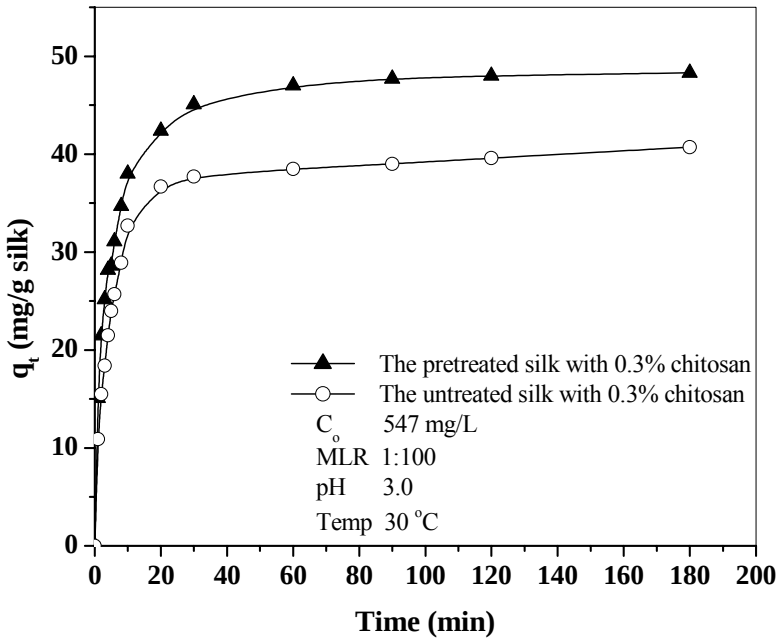


Figure 3.16 Comparison of the amount of dye adsorbed on pretreated and untreated silk with chitosan; dyeing at an initial dye concentration of 547 mg/L, MLR 1:100, pH 3.0 and 30 ^{o}C.

the carbonyl peak in silk fibroin was shifted to a longer wavelength due to hydrogen bond formation between the amino groups of chitosan and the carbonyl groups of silk fibroin (Park *et al*., 2000). The remaining free amino groups ($–NH_2$) groups on

chitosan are protonated under acidic conditions but could interact with the lac dye molecules via hydrogen bonding and ion-dipole interaction. Similar observations were reported for the dyeing of acid, direct and disperse dyes onto pretreated tussah silk fabric with chitosan (Kako and Katayama, 1997). In the study by Kako and Katayama, the tussah silk fabric was treated with chitosan by a 2 dip-2 dip method and then heated at 150 ^{o}C for 10 min. These results showed that the dye uptake of acid, direct and disperse dyes were increased in proportion to the chitosan added.

Desorption of lac dye from the pretreated silk with a 0.3% (v/v) aqueous chitosan compared to the untreated silk was also investigated. The dye bath was replaced with deionized water over a time range of 181-420 min. The amount of the dye desorbed from the silk (q_{de}) in both was calculated and then subtracted from the amount of the dye adsorbed at the equilibrium time (q_e). The amount of dye adsorbed on the untreated and pretreated silk with a 0.3% (v/v) aqueous chitosan after desorption over the time range of 181-420 min. is listed in Tables 3.15 and 3.16 respectively. As seen from Figure 3.17, the pretreatment of silk yarn with a 0.3% (v/v) aqueous solution of chitosan enhanced the dye adsorption and also decreased the dye desorption from fibres compared to the situation with untreated silk yarn. The results supported the fact that the silk fibroin chains form strong hydrogen bonds with chitosan and use the free amino groups of chitosan to hold the lac dye. It is also of interest to note that the pretreatment of silk fabric with chitosan has been reported to exhibit strong antimicrobial activity (Park *et al*., 2000; Sakurada, 1995).

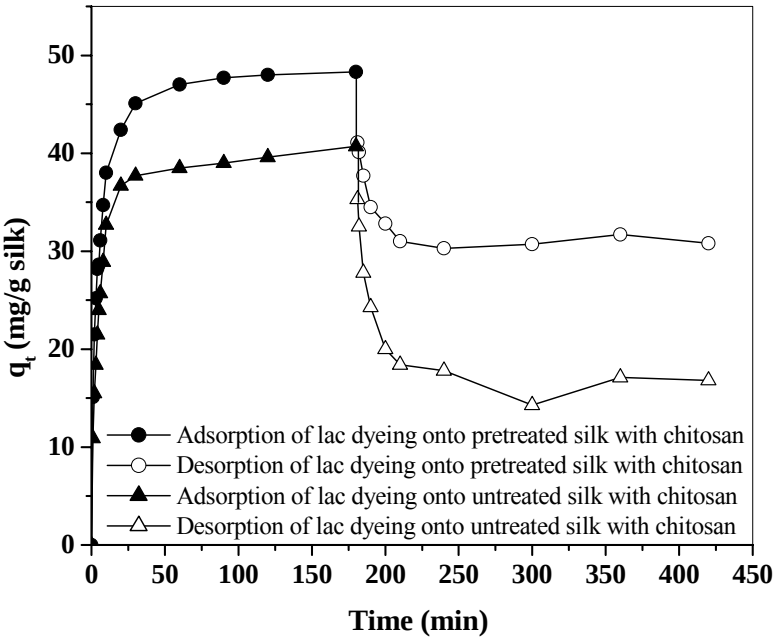


Figure 3.17 Effect of chitosan on adsorption-desorption of lac dye onto silk.

3.4 Conclusions

The adsorption isotherm and kinetics of lac dyeing onto silk were studied in this work. The most important parameters include pH, the initial dye concentration, the material to liquor ratio (MLR) and temperature, which all influence the dyeing process and hence were investigated. It was found that the adsorption capacity was dependent on the pH of the dye solution and optimal uptake on silk occurred at pH 3.0-3.5. The initial dye adsorption rate of lac dye on silk yarn was very fast with an increase in adsorption rate observed with an increase in temperature. Before equilibrium was reached, an increase in temperature led to an increase in the initial dye adsorption rate which indicated a kinetically controlled process. The experimental data fitted well to the Langmuir and Freundlich isotherms with a high correlation coefficient (R^2). A pseudo second-order kinetic model was indicated with an activation energy of 47.0 kJ/mol. This suggested that the overall rate of lac dye adsorption is likely to be controlled by the chemical process. The values of the enthalpy ($\Delta H^{\#}$) and entropy of activation ($\Delta S^{\#}$) were 44.5 kJ/mol and -143.0 J/mol K respectively. The free energy of activation ($\Delta G^{\#}$) at 30 ^{o}C was 87.8 kJ/mol. The activation parameters with and without erythrolaccin in the lac dye were similar, which is consistent with erythrolaccin only having a slight effect on the lac dyeing of silk. The free energy (ΔG^{o}), enthalpy (ΔH^{o}) and entropy (ΔS^{o}) terms for lac dyeing were also determined, and the negative values of ΔG^{o} and ΔH^{o} obtained indicated that the lac dye adsorption process is a spontaneous and an exothermic one.

The effect of chitosan treated on silk was studied. It was found that the pretreatment of silk with chitosan increased the adsorption ability of lac dye onto silk yarn compared to untreated silk under the same dyeing conditions. Also, chitosan

reduced the dye desorbed from silk yarn compared to untreated silk. This supported the fact that the silk fibroin chains form strong hydrogen bonds with chitosan (Chen *et al*., 1997; Kweon *et al*., 2001) and use the free amino groups of chitosan to adsorb the lac dye.

CHAPTER IV

ADSORPTION KINETIC AND THERMODYNAMIC STUDY OF LAC DYEING ON COTTON

4.1 Introduction

Cotton is an important textile fibre which accounts for half of the world's consumption of fibres (Horrocks and Anand, 2000). It consists of practically pure cellulose (about 88-96%) (Lewis, 1993). Cotton may be chemically described as poly (1,4-β-D-anhydroglucopyranose) (Karmakar, 1999) as shown in Figure 4.1.

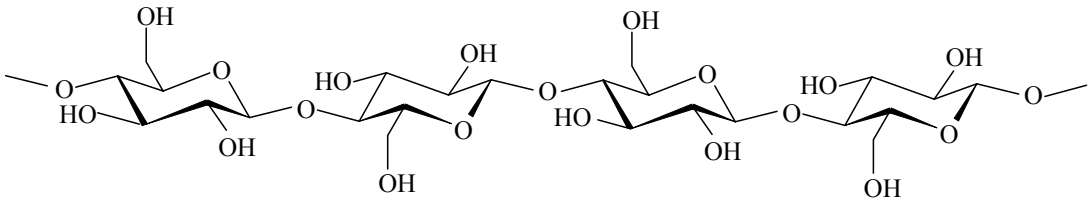


Figure 4.1 The chemical structure of cellulose.

Many studies have been undertaken to investigate the kinetic and thermodynamic of cotton dyeing with synthetic and natural dyes. The kinetic and thermodynamic sorption of Ramazol Brilliant Blue R on bleached cotton at different temperatures were reported (Gonzalez-Caballero, Epinosa-Jimenez and Gonzalez

Fernandez, 1988). The results indicated that increasing the temperature during the dyeing process increased the dye uptake on fibres. The exponential time-sorption isotherms used in their study were represented by the exponential kinetic equation $q_t = q_e (1\text{-}e^{-kt})$, where q_t and q_e referring to the amounts of reactive dye taken up by the cellulosic fibre at time t and at equilibrium respectively and k referring to the rate constant. The rate constant (k) increased with temperature. The sorption equilibrium agreed with the Freundlich isotherm.

The adsorption of direct dyes was investigated by Chrastil (1990). The diffusion equation was used to describe the time kinetics of dyeing cotton fabrics with these dyes from finite baths under different conditions. It was shown that the specific rate constants and the diffusion coefficients are dependent on the chemical structure of direct dyes. The adsorption kinetic of Direct Red 81 on untreated and crosslinked cotton fabrics from finite baths was also studied under different conditions (Hsiung and Chen, 1997). The crosslinked cotton was prepared by treating cotton with an aqueous solution of 4% 1,3-dimethylol-4,5-dihydroxyethylene urea (DMDHEU, Figure 4.2) and 1.2% $MgCl_2 \cdot 6H_2O$. The adsorption data were also fitted using the diffusion equation. It was found that the crosslinking decreased the electrical coefficients, dyeing rate constants and activation energy, but increased the structural coefficients.

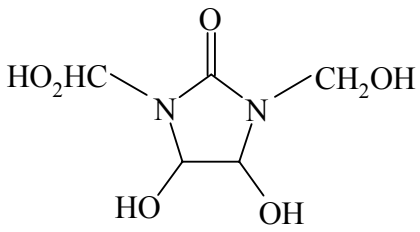


Figure 4.2 The chemical structure of 1,3-dimethylol-4,5-dihydroxyethylene urea (DMDHEU).

In addition, J. C. Chen and C. C. Chen (2000) studied the kinetic of a basic dye on untreated, crosslinked, and sulfonated/crosslinked cotton fabrics using the diffusion equation. They reported that the dye adsorption of crosslinked cotton was higher than that of untreated cotton. However, the dye adsorption of crosslinked cotton was slightly lower than that of the sulfonated/crosslinked fabric at the same dyeing duration. Furthermore, the dyeing kinetics from a finite bath of direct dyes on cotton fabrics crosslinked with various carboxylic acid groups, containing crosslinking agents were investigated using the diffusion equation (J. C. Chen, Yao, C. H. Chen and C. C. Chen, 2002). They found that the adsorption of treated cotton increased with an increase in the number of carboxylic acid groups on cotton. The heterogeneous kinetic of the uptake of a dichlorotriazinyl reactive anthraquinone dye on a cotton cloth under alkaline conditions was studied using a novel electrochemical method (Comption and Wilson, 1990). The results showed that the kinetics of dyeing is controlled by a surface process which is first order with respect to the surface concentration of dye.

The dyeing kinetics of dichlorotriazinyl-reactive dye and Procion Blue MX-R, with knitted cotton fabrics were investigated using a versatile technique based on a spectrochemical channel flow cell (Tam *et al*., 1997). It was shown that dye fixation to the fabric is controlled by a solid-liquid interfacial process that is the first order with respect to the surface concentration of dye. The mechanism of dye fixation on the knitted cotton fabrics involved the formation of a covalent bond between a hydroxy group, under alkaline conditions in the fabric, and the dye molecules, by nucleophilic displacement of chloride.

As mentioned in chapter 1, there are several classes of dye including direct, azoic, vat and reactive dyes, which can be successfully applied to cotton fibres. In general, natural cellulose fibres carry a small negative charge ($\zeta_{plateau}$ = -11 mV) due to the presence of carboxyl and hydroxyl groups (Stana-Kleinschek and Ribitsch, 1998). At a pH higher than 8, some of the hydroxyl groups on hydroxymethyl side chain can be ionized increasing the negative charge significantly (Carr, 1995). The negative charges on the surface of cellulose repel anionic dyes and hence the efficiency of dye fixation on cellulosic fibres is generally low. To counter this problem, over the years a number of studies on cotton dyeing have been carried out to improve the dye uptake and fastness properties. Most research focus is on introducing cationic sites to the cotton fabrics for interactions with anionic dyes. Kamel, Youssef and Shokry (1999) treated the cotton with cationic agents, namely 1,1-dimethyl-3-hydroxy-azetidinium chloride (DMA-AC), 1,1-diethyl-3-hydroxy-azetidinium chloride (DEA-AC) and Sandene. The treated cottons were dyed with direct dye. The results showed that the modification of cotton with tertiary or quaternary amino groups enhanced the exhaustion and fixation of direct dye on cotton. A new fibre

modification technique based on a cationic acrylic copolymer, commercially named Polymer PL was also applied in the dyeing of cotton with a reactive dye (Cai, Pailthorpe and David, 1999). It was found that pretreatment of cellulosic fibres with Polymer PL improved the dyeing ability of reactive dyes with cellulosic fabrics. In addition, Berberine, a natural cationic colorant, exhibits low exhaustion towards cellulose fibres (Kim, Yoon and Son, 2004). Therefore, the cotton fabrics were pretreated with the anionic agent (sodium, 4-(4,6-dichloro-1,3,5-triazinylamino)-benzenesulfonate), and then were dyed with Berberine. It was found that the exhaustion of Berberine onto the cotton fibres after pretreatment with the anionic agent was 23 times higher than that of the untreated sample.

The use of dendrimers to modify the dyeing behaviour of reactive dyes on cotton was carried out in the absence of both electrolyte and alkali (Burkinshaw, Mignanelli, Froehling and Bide, 2000). Dendrimers are highly branched compounds and one example is shown in Figure 4.3. It indicated that the pretreatment of cotton fabrics with dendrimers before dyeing with reactive dyes enhanced colour strength. This was mainly due to protonation of the primary and tertiary amine groups of the dendrimers under low pH. The dendrimers should act as a primary point of attraction for the anionic dye molecules. When the pH was increased during the dyeing process, the amines were deprotonated and the liberated primary amine groups can serve as highly reactive nucleophilic sites for the dye. Sodium benzoylthioglycollate is the chemical compound which was applied to modify the cotton fabrics (Broadbent and Lewis, 2000). It was found that the disperse dyeing onto the modified cotton resulted in a high colour yield and also provided a good resistance to wash fastness test.

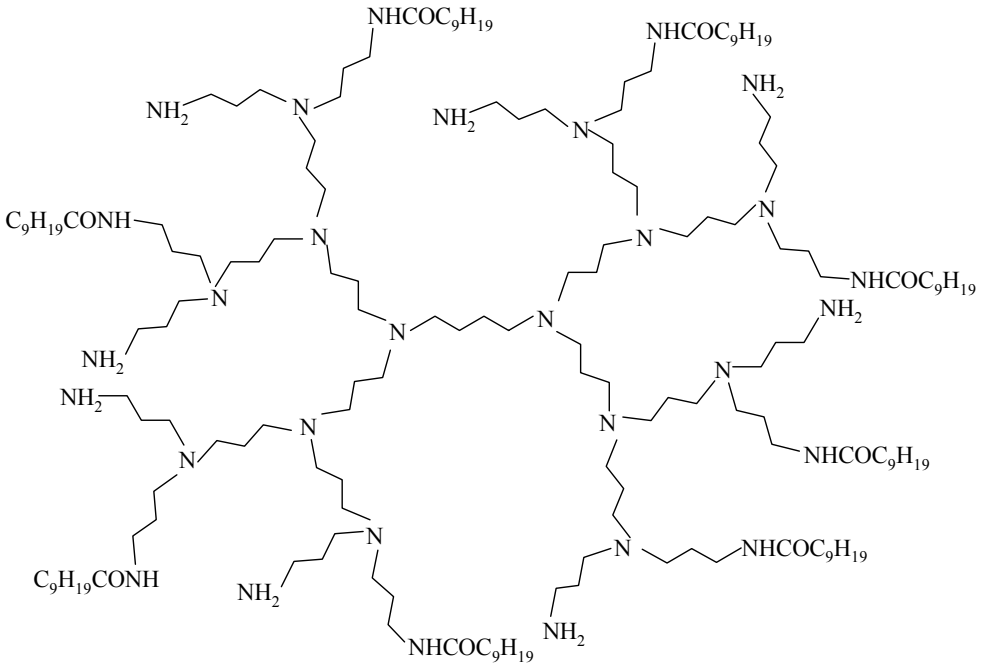


Figure 4.3 The chemical structure of a dendrimer.

In addition, some studies have been reported on the pretreatment of cotton fabrics with enzymes. For instance, the enzymes cellulase and α-amylase were used to treat the cotton fabrics before dyeing with natural dyes, chlorophyll and carmine (Tsatsaroni and Liakopoulou-Kyriakides, 1995). It was found that the enzymatic treatment of cotton increased the dye uptake of all samples compared with untreated samples and did not affect their fastness properties. In addition, they used the enzymes, namely α-amylase and amyloglycosidase, to treat the cotton fabrics before dyeing with two natural yellow pigments (Tsatsaroni, Liakopoulou-Kyriakides and Eleftheriadis, 1998). The results showed that the pretreatment with enzymes increased the dye adsorbed on the fabrics. Furthermore, the enzyme α-amylase was also used to

treat the cotton before dyeing with natural pigments extracted from Crocus sativus stigmas (Liakopoulou-Kyriakides, Tsatsaroni, Laderos and Georgiadou, 1998).

As mentioned in chapter 1, lac dye is used as a natural red dyestuff for cotton and silk dyeing in the north and the northeast of Thailand (Moeyes, 1993). However, lac dye has a small affinity for cotton because cotton does not have any cationic sites for the attachment (Saxena *et al*., 1997). An alternative way to overcome this problem is the pretreatment of cotton by using cationic agents. Rastogi *et al*. (2000) created affinity in cotton for lac dye by introducing cationic sites in the fibre. Cotton fabrics were treated with a cationic agent, Discofix DBA and then were dyed with lac dye. It was found that the cationised cotton which was dyed with lac dye exhibited a good colour yield and wet fastness properties even without mordanting. In addition, the poly (ethyleneimine) (PEI) was used as a cationic agent in cotton dyeing with lac dye (Janhom, Griffiths, R. Watanesk and S. Watanesk, 2004). It was found that PEI increased the dye adsorbed on cotton and also decreased the dye desorption from fibres.

Chitosan is a deacetylated derivative of chitin, a natural polymer found in the shell of crabs and shrimps (Majeti and Ravi, 2000). Structurally, chitosan contains two main functional groups, namely hydroxy and amino groups, as well as ether linkages (Figure 4.4). It can be used successfully as binder in the dyeing of cellulosic fabrics. According to Rippon (1984), the pretreatment of cotton fabrics with chitosan increased the exhaustion of direct dyes. In addition, chitosan was applied in textile printing (Arab-Bahmani, East and Holme, 2000). It was found that the pretreated polyester and polyester-cotton with chitosan improved the colour fastness in

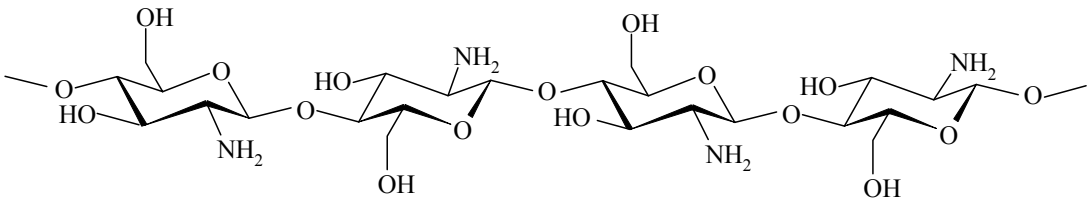


Figure 4.4 The chemical structure of chitosan.

washing, rubbing and light exposure. Chitosan was also used to treat the cotton in lac dyeing processes (Saxena *et al*., 1997). It was reported that chitosan enhanced the dye uptake of lac dye and caused lac dye sorption on cotton. This can be explained by the fact that chitosan molecules interact with cellulose molecules by some sort of bond through its amino ($-NH_2$) and hydroxy (−OH) groups. The remaining free amino acid ($-NH_2$) groups on chitosan molecules bonded to the lac dye molecules. The wash fastness of the treated cotton was improved by treatment with a crosslinking agent, dimethyloldihydroxyethylene urea. It may be due to the crosslinking agent forming a crosslink with both cellulose and chitosan.

It has been reported that the adsorption isotherm of cotton dyeing with lac dye follows the Langmuir-type isotherm at low concentration and low temperature without giving the thermodynamic parameters (Janhom *et al*., 2004). However, the kinetic and thermodynamic parameters of lac dye on cotton have not been reported previously. It is worthwhile to study the kinetic or thermodynamic of lac dye on cotton. In this work, we investigated the kinetics and thermodynamics of lac dye on cotton. Also, the effect of sodium chloride (NaCl) in dyeing process with and without pH control was studied. In addition, the pretreatment of cotton with chitosan was applied in the dyeing process. The adsorption isotherm of lac dye on pretreated cotton

with chitosan was also investigated. The scientific results from this study will hopefully give better understanding of adsorption mechanism on this dyeing process.

4.2 Experimental

4.2.1 Chemicals and materials

(a) Cotton yarn (no. 20) from villagers live in Muangphon, Khon Kaen Province

(b) Stick lac from Nong-Ying shop, Muang District, Surin Province

(c) Glacial acetic acid, CH_3COOH, analytical reagent grade, Merck Company, England

(d) Soap, commercial grade, Thailand

(e) Sodium chloride, reagent grade, Merck Company, Germany

(f) Sodium carbonate, reagent grade, Merck Company, Germany

(g) Chitosan, medium molecular weight, viscosity 200.000 cps, CAS 9012-76-4, FW 161), Aldrich Chemical Company, Germany

4.2.2 Instruments

(a) A Cary 1E UV-Visible spectrophotometer was employed for absorbance measurements using quartz cells of path length 1 cm.

(b) A pH meter (Mettler Delta 320, UK) was used to measure the pH values of the lac dye solutions.

(c) A thermostatted shaker bath (Heto-Holten A/S Denmark, Type SBD-50 cold), operated at 75 rpm, was used to study the adsorption kinetic and thermodynamic of lac dyeing onto cotton.

(d) Freeze dyer (Heto FD3 model S/N 492497-B, Cat No. 837107, Denmark) was used to dry the crude extract.

(e) Rotary evaporator (Büchi Rotavaor R-114) was employed to remove the water from the crude extract.

4.2.3 Experimental methods

4.2.3.1 Cotton yarn preparation

The cotton yarn used was purchased from villagers living in Muangphon District, Khon Kaen, Thailand. To remove the wax and impurities, the cotton yarn (100 g) was added to boiling water (2 L) which had been added soap flakes (*ca* 7 g) and sodium carbonate (3 g). The mixture was then boiled for 1 hour. The cotton was then removed, washed with hot water and cold water in order to avoid break down of the emulsion and precipitation of the impurities onto the cotton, squeezed to remove excess liquor and air dried. Finally, it was treated with 1M HCl (*ca* 2 L) at room temperature for 30 minutes and then removed and washed with deionized water until the rinsed water was neutral. The cotton yarn was then dried at room temperature.

4.2.3.2 Preparation of lac dye

Stick lac (500.90 g) from the Rain tree, *Samanea saman* (Jacq.) Merr. (*Pithecolobium saman*, Mimosaceae), in northeast Thailand (Nakhon Ratchasima) was finely powdered (18 mesh) in a grinding mill. The powdered material was extracted with deionized water (1.5 L) at 60 ^{o}C for 1 hour. The aqueous solution was filtered, the filtrate concentrated under reduced pressure (rotary evaporator) and then

dried by using a Freeze dryer to give a crude lac dye extract (36.07 g), which was then used without further purification.

4.2.3.3 Cotton pretreatment with chitosan

Chitosan (medium molecular weight, viscosity 200.000 cps, CAS 9012-76-4, FW 161) was purchased from the Aldrich Chemical Company. A 1% (w/v) stock solution of chitosan was prepared by dissolving the required amount of chitosan in a 1% (v/v) aqueous acetic acid solution. The cotton yarn (50 g), prepared as noted in section 4.2.3.1, was then immersed directly in 0.3% and 0.6% (v/v) aqueous solutions of chitosan (2 L) (prepared from the stock solution) at room temperature for 1 hour. After this time the yarn was removed and dried at 100 ^{o}C for 30 min and cured at 160 ^{o}C for 10 min. The cotton yarn, after pre-treatment with chitosan, was rinsed with water at 40 ^{o}C and allowed to dry in the open air in the laboratory.

4.2.3.4 Batch kinetic experiments

Lac dye was dissolved in deionized water to the required concentrations. The pH of the dye solutions was adjusted to 3.0 with glacial acetic acid. The dye solution (50 mL) in each conical flask (125 mL) was shaken in a thermostatted shaker bath operated at 75 rpm. After 30 minutes, the cotton yarn (0.50 g), which had been pre-warmed in the thermostatted bath for 30 minutes, was immersed in the dye solution. The cotton samples were then rapidly withdrawn after different immersion times. Dye concentrations were determined at time zero and at subsequent times using a calibration curve based on absorbance at λ_{max} 487 nm (Cary 1E UV-Visible spectrophotometer) versus dye concentration in standard lac dye solutions. The amount of dye adsorbed per gram of cotton (q_t) (mg/g cotton) at any time was calculated by a mass-balance relationship equation (4.1) as follows:

$$q_t = (C_o - C_t)\frac{V}{W} \qquad (4.1)$$

where C_o and C_t are the initial and dye concentrations (mg/L) after dyeing time t respectively. V is the volume of dye solution (mL) and W is the weight of cotton yarn (g) used. Kinetic experiments of lac dye on cotton were repeated three times.

4.2.3.5 The effect of sodium chloride on the adsorption of lac dye on cotton with and without pH control

The required dye solution in the presence of sodium chloride over concentrations range 4.30×10^{-3} - 17.1×10^{-1} M at pH 2.5, 3.0, 3.5 and without pH control were freshly prepared in deionized water. The experiments were carried out by shaking cotton yarn (0.5 g) with dye solution (50 mL), containing the different salt concentrations, in a conical flask (125 mL) at 30 ^{o}C in a thermostatted shaker bath operated at 75 rpm. The absorbance of lac dye solution for each dyeing time was monitored by UV-Visible absorption spectroscopy (Cary 1E UV-Visible spectrophotometer) until the absorbance values at λ_{max} 487 nm remained constant. The initial and equilibrium dye concentrations were determined using a calibration curve based on absorbance at λ_{max} 487 nm versus dye concentration in standard lac dye solutions. Equation (4.2) was used to calculate the amount of dye adsorbed at equilibrium (q_e) (mg/g cotton).

$$q_e = (C_o - C_e)\frac{V}{W} \qquad (4.2)$$

In equation (4.2) C_o and C_e are the initial and equilibrium dye solution concentrations (mg/L) respectively, and V is the volume of the dye solution (mL) and W is the weight of cotton yarn (g) used. The experiments in this section were repeated three times.

4.2.3.6 Batch equilibrium experiments

Different lac dye concentrations were freshly prepared in deionized water. The pH of the dye solution was adjusted to 3.0 with glacial acetic acid. The experiments were carried out by shaking cotton yarn (0.5 g) with different concentrations of dye solution (50 mL) in a conical flask (125 mL) at 10, 30 and 60 ^{o}C in a thermostatted shaker bath operated at 75 rpm. The amount of dye in the solution was monitored by UV-Visible absorption spectroscopy (Cary 1E UV-Visible spectrophotometer) until the absorbance values at λ_{max} 487 nm remained constant. The initial and equilibrium dye concentrations were determined using a calibration curve based on absorbance at λ_{max} 487 nm versus dye concentration in standard lac dye solutions. According to the equation (4.2), the amount of dye adsorbed at equilibrium (q_e) was calculated.

The influence of sodium chloride on the adsorption of lac dye on cotton was investigated in a similar manner. The cotton yarn (0.5 g) was dyed with different dye concentrations (50 mL) in the presence of 0.5 M sodium chloride at 10 and 30 ^{o}C. The pH of the dye solution before and after dyeing was measured in each experiment. The absorbance of the dye solution was measured at λ_{max} 487 nm. Equilibrium experiments of lac dye on cotton were repeated three times.

4.2.3.7 Adsorption and desorption study of lac dye on pretreated and untreated cotton with chitosan, and on cotton in the presence of 0.5 M NaCl without pH control

The cotton yarn, prepared as noted in section 4.2.3.1, with and without the pretreatment with a 0.3% (v/v) of aqueous solution of chitosan was dyed with an initial dye concentration of 473 mg/L in a thermostatted shaker bath operated at 75 rpm. The adsorption conditions are at pH 3.0, MLR of 1:100 and 30 ^{o}C. The absorbance of the dye solutions was monitored until constant absorbance values were obtained. The dyed cotton samples were then taken out and then dried at room temperature. To study the desorption of lac dye at 30 ^{o}C, deionized water (50 mL) in each conical flask (125 mL) was shaken in a thermostatted shaker bath operated at 75 rpm. After 30 minutes, the dried cotton sample (0.50 g), which had been pre-warmed in the thermostatted bath at 30 ^{o}C for 30 minutes, was immersed in deionized water. The dried cotton samples were then rapidly withdrawn after different immersion times. The desorbed dye concentrations (q_{de}) were determined using a calibration curve based on absorbance at λ_{max} 487 nm (Cary 1E UV-Visible spectrophotometer) versus dye concentration in standard lac dye solutions. The amount of dye adsorbed on cotton after desorption was calculated by subtraction.

The adsorption and desorption of lac dyeing onto cotton (initial dye concentration of 479 mg/L) in the presence of 0.5 M NaCl was investigated in a similar way. The absorbance of the dye solution was measured at λ_{max} 487 nm to monitor the amount of lac dye adsorbed and desorbed from the cotton respectively.

4.3 Results and discussion

4.3.1 Optimal conditions for lac dyeing of cotton

Experiments were undertaken to study the influence of pH, material to liquor ratio (MLR), contact time, initial dye concentration and temperature on the adsorption of lac dye on cotton. These results were reported in this chapter.

4.3.1.1 The effect of pH on the adsorption of lac dye on cotton

The pH of a dye bath is an important influencing factor for the adsorption of lac dye on cotton. In this study, the pH of lac dye solution was varied in the pH range of 2.0-5.2 by adjustment with glacial acetic acid. The adsorption data of lac dye on cotton at different pH values are listed in Table 4.1. Figure 4.5 shows the effect of pH on the adsorption of lac dye on cotton at 30 ^{o}C, with an initial dye concentration of 430 mg/L and a material to liquor ratio (MLR) of 1:100. The amount of the dye adsorbed on the cotton increased with decreasing the pH from 4.0-2.5, but dropped gradually at pH values lower than 2.5. Cotton fibre consists of a polymer of 1,4-anhydroglucoside units (Carr, 1995). Some of the pendant hydroxymethyl (-CH_2OH) groups are naturally oxidized to carboxylic acid groups during growth or subsequent processing (Carr, 1995). Due to the presence of a small number of carboxylic acid groups along the polymer chain, cellulosic fibres generally carry a weak negative charge in a dye bath with a pH higher than 4 (Carr, 1995). If the dye bath pH is raised above 8, some of the hydroxyl groups present on the hydroxymethyl side chain ionize, increasing the negative charge significantly. The negative charges on the cellulosic fibre surface repel the laccaic acid anion at pH a higher than 4.0. Therefore, the negative charge on the adsorbing surface of cellulose clearly creates a very unfavourable situation for the adsorption of lac dye on cotton at pH higher than

4.0. At a pH lower than 4.0, carboxyl and hydroxyl groups along the polymer chain of cellulosic fibres are hardly ionized. The dye molecules (Chairat, V. Rattanaphani, Bremner, S. Rattanaphani and Perkins, 2004) can reach the surface of the cotton and where they can interact with the cotton fibres via hydrogen bonding together with ion-dipole interactions. The highest adsorption capacity was observed at pH 2.5. Most organic acids in dilute solution have little effect on cotton, except oxalic, citric and tartaric acids which are liable to cause tendering (Karmakar, 1999). However, degradation of cotton may arise from acid hydrolysis of fibres (Karmakar, 1999). Therefore, the pH of the dye solution in all experiments on the adsorption kinetics was fixed at 3.0.

Table 4.1 The amount of dye adsorbed per gram of cotton at different pH; dyeing at MLR 1:100, an initial dye concentration of 430 mg/L, 30 ^{o}C and 2 hours of contact time

pH	Weight cotton (g)	A_o	$A_{t = 2\ hrs}$	q_e (mg/g cotton)
2.0	0.5024	1.7090	1.5348	4.19
2.5	0.5006	1.7745	1.5631	5.10
3.0	0.5041	1.7995	1.5979	4.83
3.5	0.5040	1.8018	1.6458	3.74
4.0	0.5000	1.7922	1.7071	2.06
4.5	0.5028	1.8122	1.7199	2.22
5.2	0.5004	1.7789	1.7255	1.29

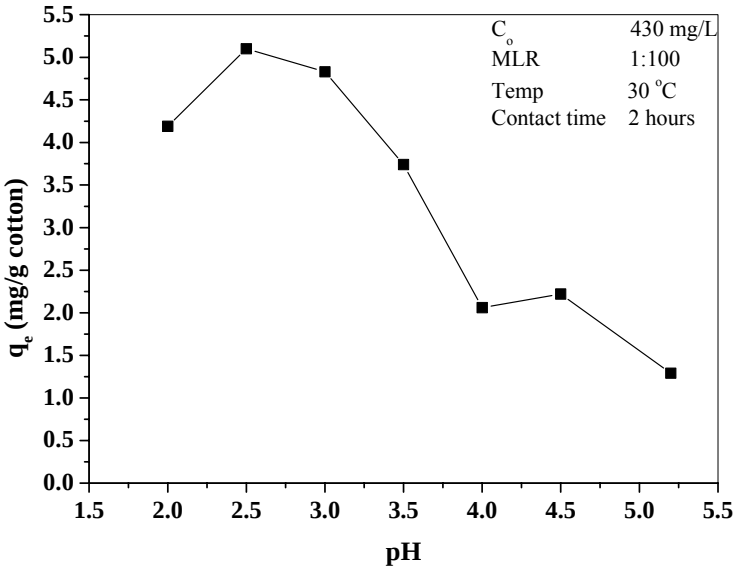


Figure 4.5 The effect of pH on the adsorption of lac dye on cotton.

4.3.1.2 The effect of material to liquor ratio (MLR) on the adsorption of lac dye on cotton

Material to liquor ratio (MLR) is another important parameter which influences the exhaustion of dye and the establishment of equilibrium between the concentration of dye on the fibre and the dye in the bath. Shorter liquor ratios shift the equilibrium in favour of the dye being on the fibre and the equilibrium is also reached more rapidly. The amount of the dye adsorbed on cotton at different material to liquor ratios is listed in Tables 4.2-4.4. It was found that MLR of 1:50, 1:100 and 1:150 showed a slightly difference in the amount of the dye adsorbed onto cotton (Figure 4.6) due to lac dye has a small affinity with cotton compared to silk yarn (Rastogi *et al*., 2000). For this reason, an increasing in the volume of dye solution showed a slightly increment in the dye adsorbed onto cotton at higher material to liquor ratio.

Therefore, the MLR of 1:100 was used for all the kinetic experiments in lac dyeing onto cotton.

Table 4.2 The amount of dye adsorbed per gram of cotton at MLR of 1:50, an initial dye concentration of 481 mg/L, pH 3.0 and 30 ^{o}C

Time (min)	**Weight cotton (g)**	**A_o**	**A_t**	**q_t (mg/g cotton)**
0	-	1.9950	-	0.00
1	0.5030	1.9950	1.8526	1.71
2	0.5019	1.9950	1.8165	2.15
4	0.5008	1.9950	1.7723	2.69
8	0.5005	1.9950	1.7367	3.12
20	0.5021	1.9950	1.6787	3.80
30	0.5000	1.9950	1.6610	4.03
60	0.5015	1.9950	1.6340	4.35
90	0.5010	1.9950	1.6080	4.66
120	0.5017	1.9950	1.6057	4.69

Table 4.3 The amount of dye adsorbed per gram of cotton at MLR of 1:100, an initial dye concentration of 480 mg/L, pH 3.0 and 30 ^{o}C

Time (min)	**Weight cotton (g)**	**A_o**	**A_t**	**q_t (mg/g cotton)**
0	-	1.9896	-	0.00
1	0.5043	1.9896	1.9247	1.55
2	0.5028	1.9896	1.9039	2.06
4	0.5077	1.9896	1.8745	2.74
8	0.5065	1.9896	1.8587	3.12
20	0.5059	1.9896	1.8200	4.05
30	0.5020	1.9896	1.8061	4.41
60	0.5082	1.9896	1.7862	4.83
90	0.5052	1.9896	1.7820	4.96
120	0.5024	1.9896	1.7817	5.00

Table 4.4 The amount of dye adsorbed per gram of cotton at MLR of 1:150, an initial dye concentration of 481 mg/L, pH 3.0 and 30 ^{o}C

Time (min)	**Weight cotton (g)**	**A_o**	**A_t**	**q_t (mg/g cotton)**
0	-	1.9950	-	0.00
1	0.5016	1.9950	1.9435	1.86
2	0.5023	1.9950	1.9350	2.16
4	0.5042	1.9950	1.9218	2.63
8	0.5061	1.9950	1.8852	3.93
20	0.5005	1.9950	1.8621	4.81
30	0.5029	1.9950	1.8538	5.09
60	0.5019	1.9950	1.8413	5.55
90	0.5001	1.9950	1.8415	5.56
120	0.5023	1.9950	1.8380	5.66

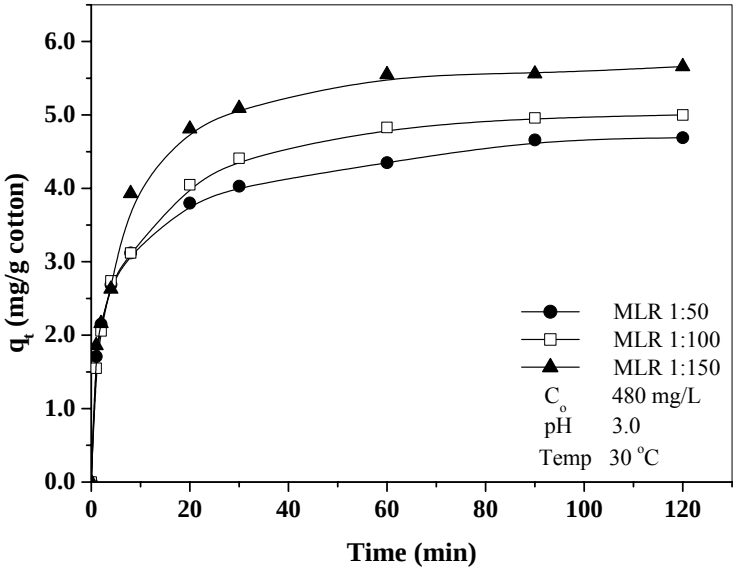


Figure 4.6 The effect of material to liquor ratio on the adsorption of lac dye on cotton.

4.3.1.3 The effect of contact time and initial dye concentration on the adsorption of lac dye on cotton

The adsorption data for lac dye on cotton at different initial dye concentrations of 137, 272 and 480 mg/L (at 30 ^{o}C, pH 3.0 and MLR 1:100) are listed in Tables 4.5-4.7. The adsorption capacity of lac dye on cotton is presented in Figure 4.7 as function of the initial dye concentration. As expected, the amount of the dye adsorbed on cotton increased with an increasing in the initial concentration of dye solution. This is due to the increase in the driving force of the concentration gradient, as an increase in the initial dye concentration (Chiou and Li, 2002). Nearly 60 minutes are required for the equilibrium adsorption for all three initial dye concentrations. Therefore, the initial dye concentration was set conservatively at 480±10 mg/L for the

kinetic study. The contact time for the adsorption isotherm study of lac dye on cotton at pH 3.0 in the section 4.3.4.1 was fixed at 2 hours.

Table 4.5 The adsorption data with an initial dye concentration of 137 mg/L, MLR 1:100, pH 3.0 and 30 ^{o}C

Time (min)	**Weight cotton (g)**	**A_o**	**A_t**	**q_t (mg/g cotton)**
0	-	0.5713	-	0.00
1	0.5030	0.5713	0.5442	0.65
2	0.5019	0.5713	0.5354	0.86
4	0.5008	0.5713	0.5248	1.12
8	0.5005	0.5713	0.5090	1.50
20	0.5021	0.5713	0.4902	1.95
30	0.5000	0.5713	0.4853	2.08
60	0.5015	0.5713	0.4758	2.30
120	0.5017	0.5713	0.4662	2.53

Table 4.6 The adsorption data with an initial dye concentration of 272 mg/L, MLR 1:100, pH 3.0 and 30 ^{o}C

Time (min)	**Weight cotton (g)**	**A_o**	**A_t**	**q_t (mg/g cotton)**
0	-	1.1304	-	0.00
1	0.5016	1.1304	1.0766	1.30
2	0.5023	1.1304	1.0649	1.57
4	0.5042	1.1304	1.0460	2.02
8	0.5061	1.1304	1.0238	2.54
20	0.5005	1.1304	0.9956	3.25
30	0.5029	1.1304	0.9901	3.37
60	0.5019	1.1304	0.9806	3.60
120	0.5023	1.1304	0.9645	3.99

Table 4.7 The adsorption data with an initial dye concentration of 480 mg/L, MLR 1:100, pH 3.0 and 30 ^{o}C

Time (min)	Weight cotton (g)	A_o	A_t	q_t (mg/g cotton)
0	-	1.9896	-	0.00
1	0.5043	1.9896	1.9247	1.55
2	0.5028	1.9896	1.9039	2.06
4	0.5077	1.9896	1.8745	2.74
8	0.5065	1.9896	1.8587	3.12
20	0.5059	1.9896	1.8200	4.05
30	0.5020	1.9896	1.8061	4.41
60	0.5082	1.9896	1.7862	4.83
90	0.5052	1.9896	1.7820	4.96
120	0.5024	1.9896	1.7817	5.00

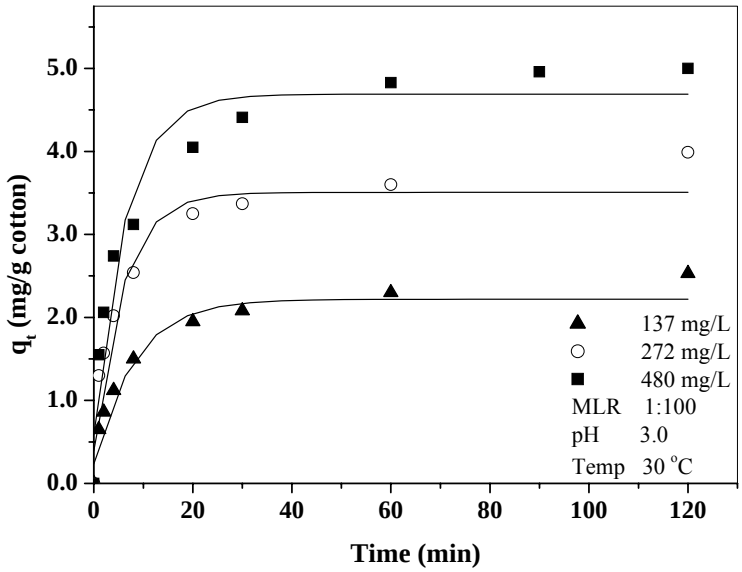


Figure 4.7 The effect of initial dye concentration on the adsorption of lac dye on cotton.

4.3.1.4 The effect of temperature on the adsorption of lac dye on cotton

The adsorption data of lac dye on cotton at 10, 30 and 60 ^{o}C are given in Tables 4.8-4.10. It was found that the higher dyeing temperature resulted in higher initial dye adsorption rates (h_i) of dye adsorption on cotton before equilibrium as shown in Figure 4.8. These results are similar to those observed for lac dyeing of silk as described in the chapter 3, section 3.3.1.4. The mobility of the large dye ions can be increased at higher temperature and subsequently the rates of dyeing can be enhanced. A similar observation was also reported in the study on the adsorption of a basic dye on the crosslinked cotton (Chen *et al*., 2000). After the equilibrium time, the dye adsorbed by the cotton decreased with an increasing the temperature, indicating an exothermic process. As can be observed from Figure 4.8 (a), the time required to reach equilibrium is shorter at higher dyeing temperature, *i.e.* 120, 60 and 5 minutes at 10, 30 and 60 ^{o}C respectively.

The optimum conditions obtained from these studies were a pH of 3.0, initial dye concentration of 480±10 mg/L and MLR of 1:100 and these conditions were subsequently used to study the kinetic adsorption of lac dye on cotton.

Table 4.8 The adsorption data at an initial dye concentration of 478 mg/L, MLR 1:100, pH 3.0 and 10 ^{o}C

Time (min)	**Weight cotton** (g)	A_o	A_t	q_t (mg/ g cotton)	q_e-q_t (mg/ g cotton)	ln(q_e-q_t)	t/q_t (g cotton min/mg)
0	-	1.9805	-	0.00	-	-	-
1	0.5047	1.9805	1.9523	0.61	4.71	1.55	1.64
2	0.5013	1.9805	1.9336	1.06	4.26	1.45	1.88
3	0.5078	1.9805	1.9213	1.34	3.98	1.38	2.24
5	0.5054	1.9805	1.9094	1.63	3.69	1.30	3.06
6	0.5080	1.9805	1.9001	1.85	3.47	1.25	3.25
7	0.5074	1.9805	1.8911	2.06	3.26	1.18	3.39
8	0.5056	1.9805	1.8840	2.24	3.08	1.13	3.57
9	0.5057	1.9805	1.8548	2.94	2.38	0.87	3.07
10	0.5024	1.9805	1.8529	3.00	2.32	0.84	3.33
12	0.5022	1.9805	1.8360	3.41	1.91	0.65	3.52
15	0.5092	1.9805	1.8354	3.38	1.94	0.66	4.44
20	0.5003	1.9805	1.8216	3.77	1.55	0.44	5.31
30	0.5025	1.9805	1.8043	4.17	1.15	0.14	7.20
60	0.5054	1.9805	1.7700	4.96	0.36	-1.03	12.09
90	0.5088	1.9805	1.7544	5.30	0.02	-3.97	16.98
120	0.5068	1.9805	1.7543	5.32	0.00	-	22.54

Table 4.9 The adsorption data at an initial dye concentration of 473 mg/L, MLR 1:100, pH 3.0 and 30 ^{o}C

Time (min)	**Weight cotton** (g)	A_o	A_t	q_t (mg/ g cotton)	q_e-q_t (mg/ g cotton)	$\ln(q_e-q_t)$	t/q_t (g cotton min/mg)
0	-	1.9619	-	0.00	-	-	-
1	0.5038	1.9619	1.9104	1.17	3.25	1.18	0.86
3	0.5062	1.9619	1.8872	1.72	2.70	0.99	1.75
4	0.5055	1.9619	1.8746	2.02	2.40	0.88	1.98
5	0.5047	1.9619	1.8736	2.05	2.37	0.86	2.44
6	0.5021	1.9619	1.8551	2.50	1.92	0.65	2.40
7	0.5020	1.9619	1.8510	2.60	1.82	0.60	2.69
8	0.5056	1.9619	1.8562	2.46	1.96	0.67	3.25
9	0.5020	1.9619	1.8496	2.64	1.78	0.58	3.42
10	0.5090	1.9619	1.8427	2.76	1.66	0.51	3.62
12	0.5028	1.9619	1.8353	2.97	1.45	0.37	4.03
15	0.5086	1.9619	1.8344	2.96	1.46	0.38	5.06
20	0.5083	1.9619	1.8211	3.28	1.14	0.13	6.10
30	0.5044	1.9619	1.8058	3.67	0.75	-0.29	8.17
60	0.5085	1.9619	1.7772	4.32	0.10	-2.31	13.89
90	0.5051	1.9619	1.7734	4.44	-	-	20.27
120	0.5021	1.9619	1.7753	4.42	-	-	27.14

Table 4.10 The adsorption data at an initial dye concentration of 470 mg/L, MLR 1:100, pH 3.0 and 60 ^{o}C

Time (min)	**Weight cotton** (g)	A_o	A_t	q_t (mg/ g cotton)	q_e-q_t (mg/ g cotton)	ln(q_e-q_t)	t/q_t (g cotton min/mg)
0	-	1.9427	-	0.00	-	-	-
1	0.5033	1.9427	1.8803	1.43	1.17	0.16	0.70
2	0.5000	1.9427	1.8646	1.82	0.78	-0.25	1.10
3	0.5088	1.9427	1.8605	1.89	0.71	-0.34	1.59
4	0.5057	1.9427	1.8537	2.06	0.54	-0.62	1.94
5	0.5092	1.9427	1.8482	2.18	0.42	-0.86	2.30
6	0.5048	1.9427	1.8527	2.09	0.51	-0.67	2.87
7	0.5040	1.9427	1.8447	2.28	0.32	-1.15	3.07
8	0.5041	1.9427	1.8473	2.22	0.38	-0.97	3.60
9	0.5014	1.9427	1.8493	2.18	0.42	-0.87	4.12
10	0.5028	1.9427	1.8476	2.22	0.38	-0.96	4.51
12	0.5075	1.9427	1.8490	2.16	0.44	-0.83	5.55
15	0.5050	1.9427	1.8409	2.37	0.23	-1.46	6.33
20	0.5064	1.9427	1.8446	2.27	0.33	-1.12	8.80
30	0.5077	1.9427	1.8408	2.36	0.24	-1.42	12.72
60	0.5081	1.9427	1.8370	2.45	0.15	-1.87	24.52
90	0.5025	1.9427	1.8312	2.61	-	-	34.48
120	0.5034	1.9427	1.8317	2.60	-	-	46.21

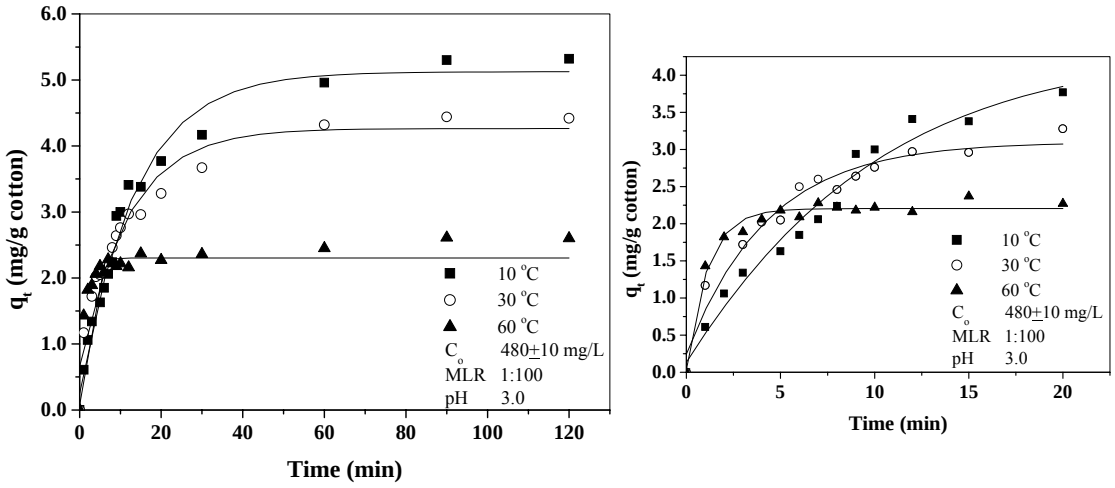


Figure 4.8 The effect of contact time and temperature of lac dye on cotton at an initial dye concentration 480±10 mg/L, MLR 1:100 and pH 3.0.

(a) The effect of contact time and temperature over a period of 0-120 min.

(b) The effect of contact time and temperature over a period of 0-20 min.

4.3.2 Kinetics of adsorption

In order to analyze the adsorption kinetics of lac dye on cotton, the pseudo first- and second-order kinetic models were used to analyze the experimental data.

Kinetic data obtained from this adsorption have been analyzed using the pseudo first-order kinetic model proposed by Lagergren (Ho and Chiang, 2001) according to the equation (3.2). These results are listed in Table 4.11. Based on the correlation coefficients, the adsorption of lac dye on cotton is not likely to be a first order reaction.

The pseudo second-order kinetic model was also used to test the experimental data using equation (3.5). Figure 4.9 shows a plot of (t/q_t) against t for the adsorption of lac dye on cotton. The slopes and intercepts of these plots were used to calculate the adsorption capacity ($q_{e,\text{cal}}$) and the rate constant (k_2). The experimental data showed a good compliance with the pseudo second-order equation and the correlation coefficients for the linear plots were higher than 0.99 for all the experimental data. Also, the calculated $q_{e,\text{cal}}$ values agreed very well with the experimental data. These results suggested that the experimental data for the adsorption kinetic of lac dye on cotton were fitted by the pseudo second-order kinetic model. A similar phenomenon was also observed in the kinetics of wool dyeing with acid dyes (Bruce and Broadwood, 2000). They reported that the uptake rate of the acid dye on wool was described by a second-order rate expression based on the formation of a protein-dye complex as the rate determining step.

Table 4.11 Comparison of the pseudo first- and second-order adsorption rate constants of lac dyeing onto cotton at an initial dye concentration 480±10 mg/L, MLR 1:100 and pH 3.0

Temp	$q_{e,\text{exp}}$	**Pseudo first-order model**		**Pseudo second-order model**			
(°C)	**(mg/g cotton)**	k_1 **(min^{-1})**	R^2	k_2 **(g cotton/mg min)**	$q_{e,\text{cal}}$ **(mg/ g cotton)**	h_i **(mg/ g cotton min)**	R^2
10	5.32	4.29x 10^{-2}	0.9803	1.60 x 10^{-2}	5.84	0.55	0.9989
30	4.42	5.52 x 10^{-2}	0.9914	3.39 x 10^{-2}	4.68	0.74	0.9990
60	2.60	2.66 x 10^{-2}	0.7893	2.30 x 10^{-1}	2.62	1.58	0.9996

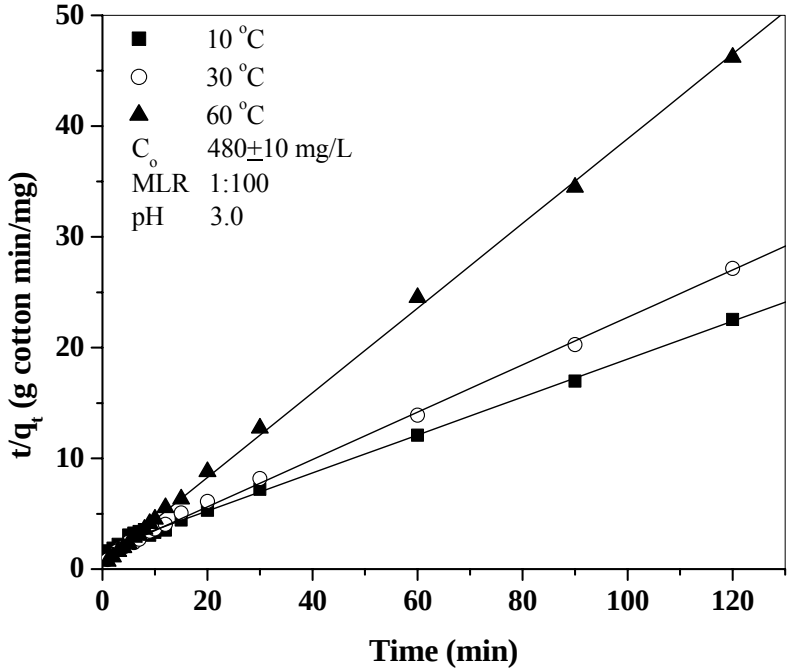


Figure 4.9 Plot of the pseudo second-order equation at different temperatures.

4.3.3 Activation parameters

From the rate constant k_2 obtained in the section 4.3.2, the activation energy (E_a) for the adsorption of lac dye on cotton was determined using the Arrhenius equation (Equation 3.8). The Arrhenius plot of ln k against 1/T for the adsorption of lac dye on cotton is shown in Figure 4.10 and the activation energy value is listed in Table 4.12.

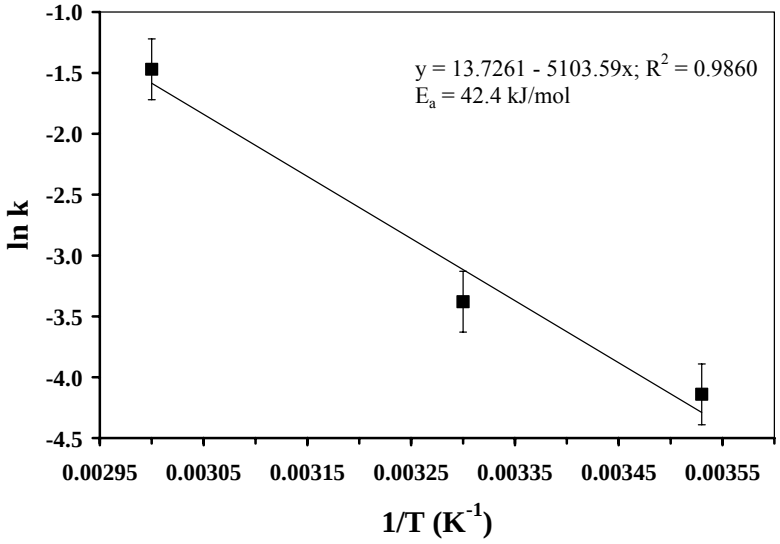


Figure 4.10 Arrhenius plot for the adsorption of lac dye on cotton.

Table 4.12 Activation parameters for the adsorption of lac dye on cotton at an initial dye concentration of 480±10 mg/L, MLR 1:100 and pH 3.0

Temp (^oC)	k_2 (g cotton/mg min)	E_a (kJ/mol)	R^2	$\Delta H^\#$ (kJ/mol)	$\Delta S^\#$ (J/mol K)	$\Delta G^\#$ (kJ/mol)	R^2
10	1.60×10^{-2}					79.3	
30	3.39×10^{-2}	42.4	0.9860	39.8	-139.7	82.1	0.9836
60	2.30×10^{-1}					86.3	

According to Eyring equation (Dogan and Alkan, 2003), free energy ($\Delta G^\#$), enthalpy ($\Delta H^\#$) and entropy ($\Delta S^\#$) of activation were calculated from the slope and intercept of a plot of ln (k/T) versus 1/T in Figure 4.11. The calculated values are listed in Table 4.12. The negative value of activation entropy ($\Delta S^\#$) suggested there is an interaction between lac dye and cotton.

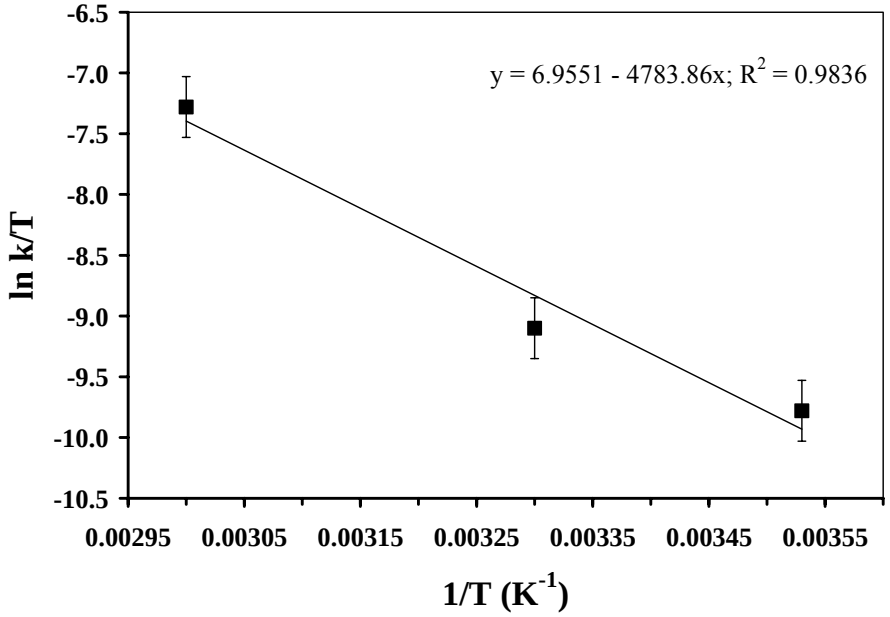


Figure 4.11 Plot of ln (*k/T*) against 1/*T* for the adsorption of lac dye on cotton.

4.3.4 Adsorption isotherms

4.3.4.1 Adsorption isotherm of lac dye on cotton at pH 3.0

The isothermal equilibrium data (Tables 4.13-4.15) of lac dye on cotton under conditions of pH 3.0, MLR 1:100 in the dye concentration range 52-1421 mg/L at 10, 30 and 60 ^{o}C were described employing the Langmuir isotherm equation as shown in Figure 4.12. It was found that the uptake of the dye decreased with increasing temperature, thereby indicating the process is exothermic. This is in agreement with the experimental results of the adsorption of lac dye on cotton without pH control by Janhom *et al*. (2004). In this experiment, the dye solutions in the concentration range 655-1421 mg/L before and after dyeing were diluted before measuring the absorbance. As seen from Figure 4.12, the adsorption isotherm of lac dye on cotton can be classified as the Langmuir isotherm.

Table 4.13 Data for the adsorption isotherm of lac dye on cotton at the initial dye concentration range 52-1421 mg/L, MLR 1:100, pH 3.0 and 10 ^{o}C

Initial conc. (mg/L)	**Weight cotton (g)**	**A_o**	**$A_{t = 2\ hrs}$**	**[Dye uptake] x dilute factor (mg/L)**	**q_e (mg/g cotton)**	**C_e (mg/mL)**
52	0.5009	0.2184	0.1625	13.5x1 = 13.5	1.35	0.0386
97	0.5050	0.4059	0.3106	23.0x1 = 23.0	2.28	0.0744
236	0.5014	0.9810	0.8365	34.9x1 = 34.9	3.48	0.2014
417	0.5032	1.7290	1.5421	45.1x1 = 45.1	4.49	0.3718
655	0.5052	1.3583	1.2272	31.7x2 = 63.4	6.27	0.5915
841	0.5044	1.7434	1.5698	41.9x2 = 83.8	8.31	0.7570
1421	0.5044	1.4734	1.3793	22.7x4 = 90.8	9.01	1.3300

Table 4.14 Data for the adsorption isotherm of lac dye on cotton at the initial dye concentration range 52-1421 mg/L, MLR 1:100, pH 3.0 and 30 ^{o}C

Initial conc. (mg/L)	**Weight cotton (g)**	**A_o**	**$A_{t = 2\ hrs}$**	**[Dye uptake] x dilute factor (mg/L)**	**q_e (mg/g cotton)**	**C_e (mg/mL)**
52	0.5044	0.2184	0.1737	10.8 = 10.8	1.07	0.0413
97	0.5045	0.4059	0.3316	17.9x1 = 17.9	1.78	0.0794
236	0.5005	0.9810	0.8414	33.7x1 = 33.7	3.37	0.2026
417	0.5018	1.7290	1.5597	40.9x1 = 40.9	4.07	0.3761
655	0.5049	1.3583	1.2512	25.9x2 = 51.8	5.12	0.6031
841	0.5023	1.7434	1.5981	35.1x2 = 70.2	6.99	0.7707
1421	0.5017	1.4734	1.3876	20.7x4 = 82.8	8.26	1.3380

Table 4.15 Data for the adsorption isotherm of lac dye on cotton at the initial dye concentration range 52-1421 mg/L, MLR 1:100, pH 3.0 and 60 ^{o}C

Initial conc. (mg/L)	**Weight cotton (g)**	**A_o**	**$A_{t = 2\ hrs}$**	**[Dye uptake] x dilute factor (mg/L)**	**q_e (mg/g cotton)**	**C_e (mg/mL)**
52	0.5018	0.2184	0.1905	6.7x1 = 6.7	0.67	0.0453
97	0.5037	0.4059	0.3580	11.6x1 = 11.6	1.15	0.0858
236	0.5013	0.9810	0.9007	19.4x1 = 19.4	1.93	0.2169
417	0.5052	1.7290	1.6365	22.3x1 = 22.3	2.21	0.3946
655	0.5036	1.3583	1.3050	12.9x2 = 25.7	2.56	0.6291
841	0.5034	1.7434	1.6974	11.1x2 = 22.2	2.21	0.8187
1189	0.5020	1.2333	1.1964	8.9x4 = 35.6	3.55	1.1533
1421	0.5006	1.4734	1.4356	9.1x4 = 36.4	3.65	1.3844

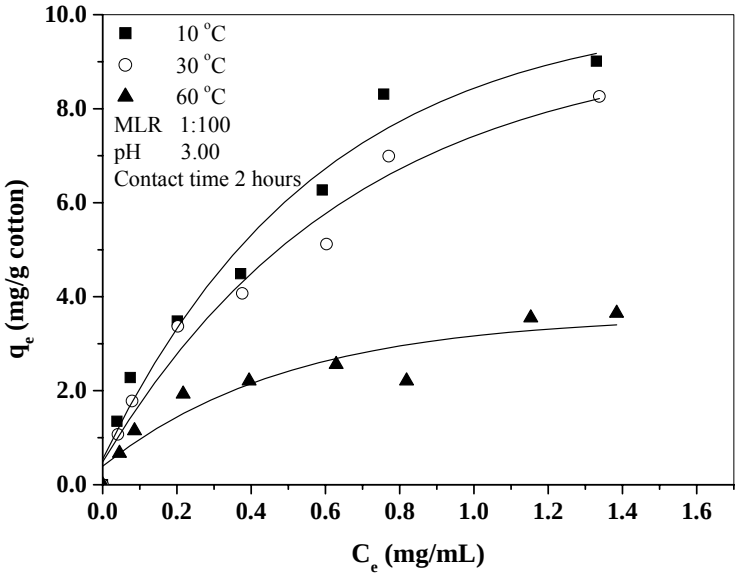


Figure 4.12 Adsorption isotherm of lac dye on cotton at pH 3.0 in the initial dye concentration range 52-1421 mg/L.

Due to the inaccurate values from dilution at the initial dye concentration higher than 650 mg/L, the adsorption isotherm of lac dye on cotton in this study was investigated in the dye concentration range 50-550 mg/L. The experimental data for the adsorption isotherm of this study over the initial dye concentration range 50-550 mg/L are listed in Tables 4.16-4.18.

Table 4.16 Data for the adsorption isotherm of lac dye on cotton at the initial dye concentration range 52-490 mg/L, MLR 1:100, pH 3.0 and 10 ^{o}C

Initial conc. (mg/L)	**Weight cotton (g)**	q_e **(mg/ g cotton)**	C_e **(mg/mL)**	$1/q_e$ **(g cotton/mg)**	$1/C_e$ **(mL/mg)**	**ln** q_e	**ln** C_e
52	0.5061	1.33	0.0382	0.7533	26.16	0.29	-3.26
107	0.5034	2.38	0.0832	0.4196	12.02	0.87	-2.49
158	0.5036	2.89	0.1287	0.3457	7.77	1.06	-2.05
209	0.5023	3.34	0.1756	0.2991	5.70	1.21	-1.74
261	0.5046	3.52	0.2258	0.2840	4.43	1.26	-1.49
378	0.5053	4.59	0.3320	0.2181	3.01	1.52	-1.10
490	0.5049	5.76	0.4314	0.1735	2.32	1.75	-0.84

Table 4.17 Data for the adsorption isotherm of lac dye on cotton at the initial dye concentration range 55-465 mg/L, MLR 1:100, pH 3.0 and 30 ^{o}C

Initial conc. (mg/L)	**Weight cotton (g)**	q_e **(mg/ g cotton)**	C_e **(mg/mL)**	$1/q_e$ **(g cotton/mg)**	$1/C_e$ **(mL/mg)**	**ln** q_e	**ln** C_e
55	0.5045	1.04	0.0443	0.9641	22.55	0.04	-3.12
108	0.5005	1.51	0.0925	0.6606	10.81	0.41	-2.38
160	0.5074	2.13	0.1389	0.4703	7.20	0.75	-1.97
215	0.5038	2.40	0.1907	0.4162	5.24	0.88	-1.66
266	0.5073	3.07	0.2346	0.3263	4.26	1.12	-1.45
368	0.5000	3.58	0.3318	0.2797	3.01	1.27	-1.10
465	0.5032	3.69	0.4280	0.2719	2.34	1.30	-0.85

Table 4.18 Data for the adsorption isotherm of lac dye on cotton at the initial dye concentration range 59-514 mg/L, MLR 1:100, pH 3.0 and 60 ^{o}C

Initial conc. (mg/L)	**Weight cotton (g)**	q_e **(mg/ g cotton)**	C_e **(mg/mL)**	$1/q_e$ **(g cotton/mg)**	$1/C_e$ **(mL/mg)**	**ln** q_e	**ln** C_e
59	0.5000	0.67	0.0481	1.4984	20.77	-0.40	-3.03
118	0.5033	1.10	0.0966	0.9073	10.36	0.10	-2.34
176	0.5029	1.39	0.1468	0.7366	6.81	0.31	-1.92
233	0.5062	1.60	0.1988	0.6262	5.03	0.47	-1.62
291	0.5023	1.87	0.2469	0.5358	4.05	0.62	-1.40
404	0.5038	2.36	0.3438	0.4242	2.91	0.86	-1.07
514	0.5016	2.58	0.4391	0.3873	2.28	0.95	-0.82

As seen from Figure 4.13, when $1/q_e$ is plotted against $1/C_e$ according to the equation (3.13) in the chapter 3 (section 3.1.3.1), the Langmuir model fitted the experimental data very well with high correlation coefficients ($R^2 > 0.98$). The values of the Langmuir constants Q and b were calculated from the intercepts and slopes of different straight lines respectively at different temperatures. The calculated results are reported in Table 4.19. It can be seen from the Table 4.19 that Q values decreased with increasing temperature. Similar observations were reported for the adsorption of silk dyeing with lac dye (Kongkachuichay *et al*., 2002).

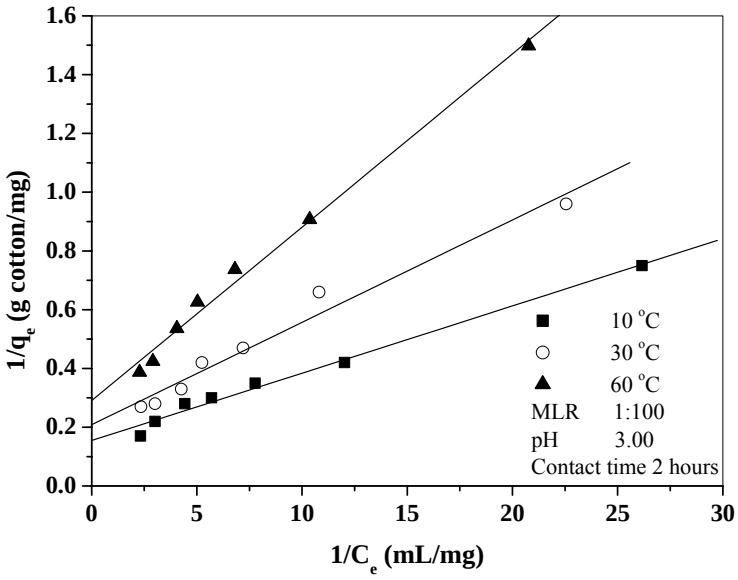


Figure 4.13 A plot of $1/q_e$ against $1/C_e$ for the adsorption of lac dye on cotton at the initial dye concentration range 50-550 mg/L, MLR 1:100 and pH 3.0.

Table 4.19 Langmuir and Freundlich isotherm constants of the adsorption of lac dye on cotton at different temperatures, the initial dye concentration range 50-550 mg/L, MLR 1:100 and pH 3.0

Temp (°C)	Langmuir			Freundlich		
	Q (mg/g cotton)	b (mL/mg)	R^2	Q_f (mg/g cotton)	n	R^2
10	6.46	6.76	0.9940	8.88	1.79	0.9914
30	4.80	5.98	0.9866	6.63	1.68	0.9915
60	3.44	4.93	0.9961	4.40	1.63	0.9979

The Langmuir constant b is related to the enthalpy of adsorption, (b α (exp ($-\Delta H^o/RT$)) (Gupta, Ali, Suhas and Mohan, 2003). Therefore, the thermodynamic parameters including the free energy (ΔG^o), enthalpy (ΔH^o), and entropy (ΔS^o) changes are also evaluated using the following equations (Jain *et al*., 2003):

$$\Delta G^o = -RT\ln(b) \tag{4.3}$$

$$\ln\left(\frac{b_2}{b_1}\right) = -\frac{\Delta H^o}{R}\left(\frac{1}{T_2} - \frac{1}{T_1}\right) \tag{4.4}$$

$$\ln(b) = \frac{\Delta S^o}{R} - \frac{\Delta H^o}{RT} \tag{4.5}$$

$$\Delta G^{o} = \Delta H^{o} - T\Delta S^{o} \qquad (4.6)$$

Enthalpy (ΔH^{o}) and entropy (ΔS^{o}) of the adsorption was calculated from the slope and intercept of a graph of ln (b) versus $1/T$ (Figure 4.14). The results are reported in Table 4.20. The negative value of the free energy (ΔG^{o}) indicated the spontaneous nature of lac dye adsorption on cotton. The change in enthalpy (ΔH^{o}) was found to be a small negative. The negative value confirms the exothermic nature of adsorption. Also, the negative value of entropy (ΔS^{o}) indicated that the decreased randomness after the adsorption of lac dye on cotton. The adsorption of lac dye on cotton in this study is similar to the adsorption of direct dyes on cotton (Chrastil, 1990). It was found that the enthalpy for the adsorption of cotton dyeing with the direct dyes, namely Yellow 29, Yellow 50, Orange 31, Red 81, Red 75, Red 23, Violet 51, and Blue 71, were in the range of 0.84 - 6.28 kJ/mol (Chrastil, 1990).

Table 4.20 Thermodynamic parameters for the adsorption of lac dye on cotton at different temperatures, MLR 1:100, the initial dye concentration range 50-550 mg/L and pH 3.0

Temp (^{o}C)	***b*** **(mL/mg)**	**ΔG^{o} (kJ/mol)**	**ΔH^{o} (kJ/mol)**	**ΔS^{o} (J/mol K)**	***R^2***
10	6.76	-4.50			
30	5.98	-4.51	-4.88	-1.31	0.9986
60	4.93	-4.42			

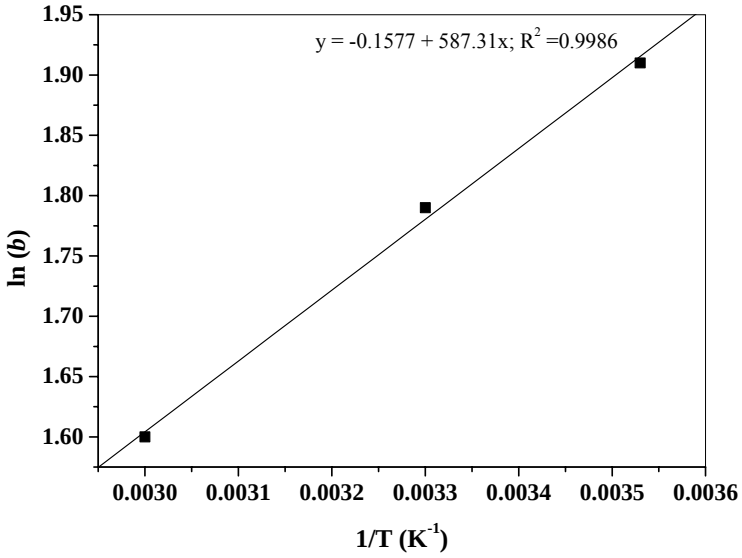


Figure 4.14 A plot of ln (*b*) against 1/*T* for the adsorption of lac dye on cotton at the initial dye concentration range 50-550 mg/L, MLR 1:100 and pH 3.0.

The Freundlich isotherm model was also used to fit the experimental data for the adsorption of lac dye on cotton at pH 3.0. Linear plots of ln q_e versus ln C_e showed the adsorption follows the Freundlich isotherm model well (Figure 4.15) with high correlation coefficients ($R^2 > 0.99$). The value of Q_f has been used as a relative measure of adsorption capacity. It was found that Q_f increased with decreasing temperature which again indicated the process was exothermic. In addition, *n* values more than 1 indicated the favorable adsorption condition. It can be concluded from these results that the adsorption of lac dye on cotton at pH 3.0 follows both the Langmuir and Freundlich isotherm models.

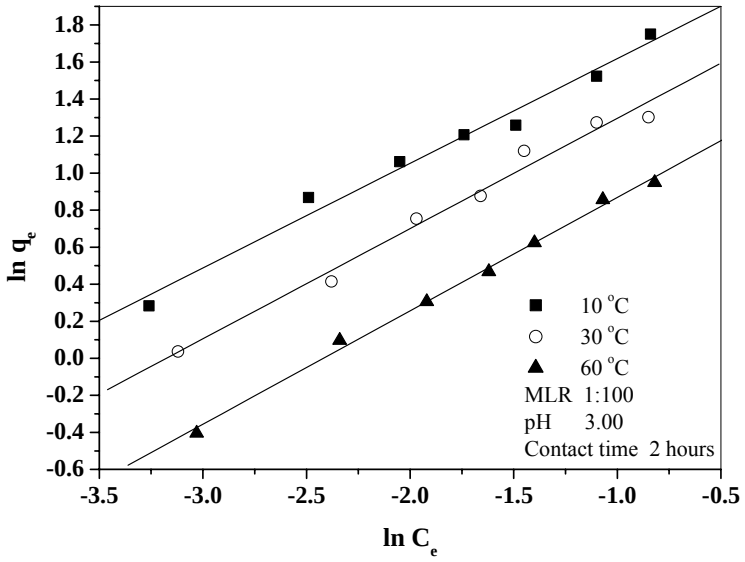


Figure 4.15 Freundlich isotherm of lac dye on cotton at the initial dye concentration range 50-550 mg/L, MLR 1:100 and pH 3.0.

4.3.4.2 The effect of sodium chloride on the adsorption of lac dye on cotton with and without pH control

Due to the presence of hydroxyl and carboxyl groups, the natural cellulose fibres carry a negative charge ($\zeta_{plateau}$ = -11 mV) (Stana-Kleinschek and Ribitsch, 1998) whereas lac dye is an acid dye (Saxena *et al*., 1997) as a result of the presence of phenolic and carboxylic acid groups in the molecule providing a negative charge. Therefore, lac dye molecules and cellulose repel one another at pH values higher than 4. This means that electrical repulsion must be overcome by other forces of attraction between the lac dye and cellulose fibres in dyeing process. For this reason, electrolytes such as sodium chloride have been added into dye baths to promote the exhaustion of anionic dye on cellulose fibres (Horrocks and Anand, 2000). In addition, many experimental results have been reported where electrolytes

such as sulfates and phosphates increased the sorption of acid dyes on nylon and wool and also increased the sorption of basic dyes on acrylic fibres if the salt concentrations are high enough (Yang, 1998). Therefore, the effect of sodium chloride (concentration range $4.30x10^{-3}$ - $17.1x10^{-1}$ M) on the adsorption of lac dye on cotton with and without pH control was investigated in this section and the results are shown in Tables 4.21-4.24.

Table 4.21 The amount of dye adsorbed per gram of cotton at different sodium chloride concentrations without pH control at an initial dye concentration of 415 mg/L, MLR 1:100 and 30 ^{o}C

[NaCl] (M)	Weight cotton (g)	pH before dyeing	pH after dyeing	A_o	$A_{t\ =\ 2\ hrs}$	q_e (mg/g cotton)
0	0.5076	5.43	6.23	1.7215	1.6575	1.46
$4.30x10^{-3}$	0.5078	5.37	6.22	1.7196	1.6663	1.20
$7.70x10^{-3}$	0.5011	5.35	6.12	1.7203	1.6657	1.25
$1.70x10^{-2}$	0.5032	5.30	5.98	1.6899	1.6533	0.81
$8.60x10^{-2}$	0.5068	5.17	5.98	1.6771	1.6179	1.34
$1.71x10^{-1}$	0.5071	5.12	5.80	1.7009	1.5800	2.81
$3.42x10^{-1}$	0.5057	4.97	5.77	1.6579	1.4581	4.71
$6.84x10^{-1}$	0.5032	4.93	5.68	1.6430	1.4076	5.58
$10.3x10^{-1}$	0.5050	4.83	5.63	1.6346	1.4018	5.50
$13.7x10^{-1}$	0.5034	4.80	5.65	1.5851	1.3441	5.72
$17.1x10^{-1}$	0.5021	4.75	5.53	1.5994	1.3333	6.33

Table 4.22 The amount of dye adsorbed per gram of cotton at different sodium chloride concentrations at pH 2.5, an initial dye concentration of 460 mg/L, MLR 1:100 and 30 ^{o}C

[NaCl] (M)	**Weight cotton (g)**	**A_o**	**$A_{t\ =\ 2\ hrs}$**	**q_e (mg/g cotton)**
0	0.5051	1.9087	1.6752	5.52
$4.30x10^{-3}$	0.5095	1.8554	1.6257	5.38
$7.70x10^{-3}$	0.5037	1.8620	1.6473	5.08
$1.70x10^{-2}$	0.5046	1.8576	1.6249	5.50
$8.60x10^{-2}$	0.5043	1.8557	1.6473	4.92
$1.71x10^{-1}$	0.5044	1.8369	1.6525	4.35
$3.42x10^{-1}$	0.5071	1.8320	1.6535	4.19
$6.84x10^{-1}$	0.5021	1.7985	1.6357	3.85
$10.3x10^{-1}$	0.5021	1.7766	1.6043	4.08
$13.7x10^{-1}$	0.5054	1.7552	1.5728	4.29
$17.1x10^{-1}$	0.5048	1.7326	1.5375	4.60

Table 4.23 The amount of dye adsorbed per gram of cotton at different sodium chloride concentrations at pH 3.0, an initial dye concentration of 454 mg/L, MLR 1:100 and 30 ^{o}C

[NaCl] (M)	**Weight cotton (g)**	**A_0**	**$A_{t = 2\ hrs}$**	**q_e (mg/g cotton)**
0	0.5063	1.8837	1.6731	4.96
$4.30x10^{-3}$	0.5084	1.8286	1.5975	5.42
$7.70x10^{-3}$	0.5058	1.8223	1.5880	5.53
$1.70x10^{-2}$	0.5040	1.8188	1.5890	5.44
$8.60x10^{-2}$	0.5051	1.8119	1.5974	5.06
$1.71x10^{-1}$	0.5003	1.8173	1.6001	5.18
$3.42x10^{-1}$	0.5058	1.7614	1.5783	4.31
$6.84x10^{-1}$	0.5059	1.7097	1.5489	3.77
$10.3x10^{-1}$	0.5018	1.7191	1.5445	4.14
$13.7x10^{-1}$	0.5027	1.6770	1.5108	3.93
$17.1x10^{-1}$	0.5027	1.6555	1.4804	4.14

Table 4.24 The amount of dye adsorbed per gram of cotton at different sodium chloride concentrations at pH 3.5, an initial dye concentration of 460 mg/L, MLR 1:100 and 30 ^{o}C

[NaCl] (M)	**Weight cotton (g)**	**A_o**	**$A_{t = 2\ hrs}$**	**q_e (mg/g cotton)**
0	0.5099	1.9087	1.7589	3.48
$4.30x10^{-3}$	0.5012	1.8884	1.7073	4.30
$7.70x10^{-3}$	0.5074	1.8620	1.7145	3.44
$1.70x10^{-2}$	0.5074	1.8797	1.7326	3.44
$8.60x10^{-2}$	0.5029	1.8615	1.7170	3.40
$1.71x10^{-1}$	0.5072	1.8493	1.7058	3.35
$3.42x10^{-1}$	0.5053	1.8317	1.6870	3.39
$6.84x10^{-1}$	0.5011	1.8080	1.6731	3.18
$10.3x10^{-1}$	0.5038	1.7892	1.6497	3.28
$13.7x10^{-1}$	0.5056	1.7733	1.6237	3.51
$17.1x10^{-1}$	0.5052	1.7547	1.6040	3.54

It was found that an increase in the sodium chloride concentration over the range $4.31x10^{-3}$ - $6.84x10^{-1}$ M led to an increase in the dye adsorbed on cotton and remained constant in the concentration range $10.3x10^{-1}$ - $17.1x10^{-1}$ M when cotton fibres were dyed with lac dye solution without pH control (Figure 4.16). Similar results were also obtained by Janhom *et al*. (2004) without giving the thermodynamic parameters.

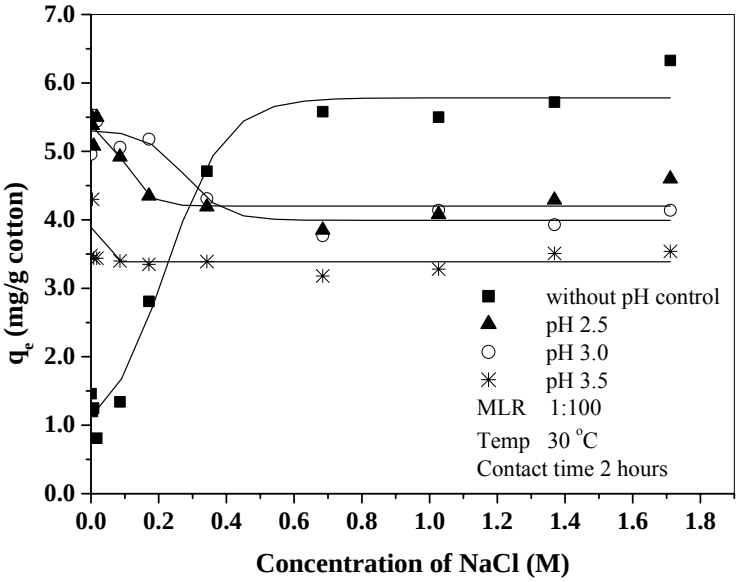


Figure 4.16 The effect of NaCl salt on the adsorption of lac dye on cotton with and without pH control (pH of the dye solutions before and after dyeing over the pH range 5.43-4.75 and 6.23-5.53 respectively).

These results can be explained by the Donnan model (Perkins, 1996) as shown in Figure 4.17. According to the Donnan model, cellulose fibres are negatively charged at a pH higher than 4 (Carr, 1995) due to the presence of the hydroxyl and carboxyl groups in molecules (Stana-Kleinschek and Ribitsch, 1998). Dyeing occurs

by transfer of dye from the external to the internal solution. Upon addition of sodium chloride (as electrolyte) into the dye solution, the sodium ions (Na^+) can distribute between the external solution and internal solution so that the negative charge on the cellulose surface is neutralized or shielded, allowing the lac dye molecules to be adsorbed on the cotton (Horrocks and Anand, 2000; Yang, 1998; Stana-Klenschek and Ribisch, 1998). However, sodium chloride has no effect on the adsorption of lac dye on cotton at pH 2.5, 3.0 and 3.5 (Figure 4.16) because carboxylic acid and hydroxyl groups rarely ionize at pH values lower than 4.0. It indicated that hydrogen ion (H^+ ion) plays a more important role than sodium ion (Na^+). The adsorption isotherm of lac dye on cotton in the presence of 0.5 M sodium chloride has been reported in the next section.

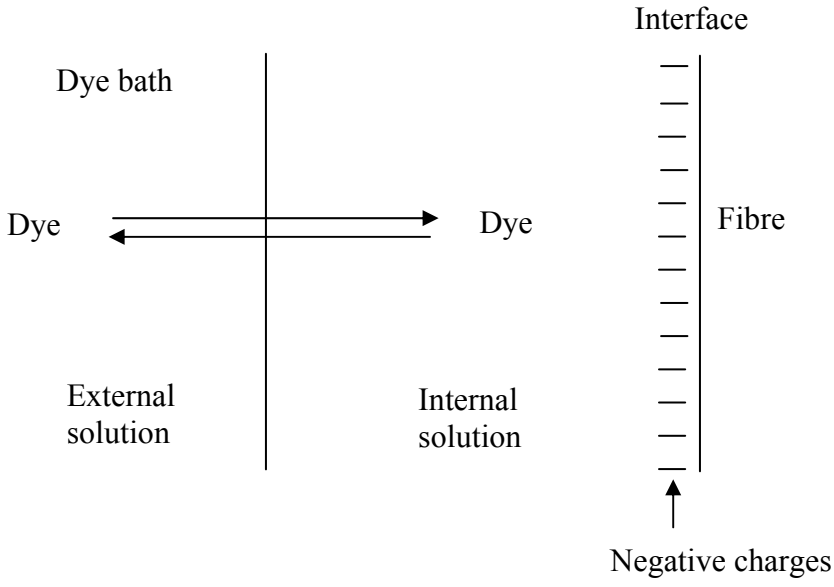


Figure 4.17 Donnan model for dye adsorption (Perkins, 1996).

4.3.4.3 Adsorption isotherm of lac dye on cotton in the presence of 0.5 M sodium chloride without pH control

The results of the adsorption data (Tables 4.25 and 4.26) were analyzed with the help of the following linear forms of the Langmuir and Freundlich isotherms according to the equations (3.13) and (3.16) respectively.

Table 4.25 Data for the adsorption isotherm of lac dye on cotton in the presence of 0.5 M sodium chloride without pH control at the initial dye concentration range 85-819 mg/L, MLR 1:100 and 10 ^{o}C; pH before and after dyeing = 5.57 and 6.32 respectively

Initial conc. (mg/L)	**Weight cotton (g)**	q_e **(mg/ g cotton)**	C_e **(mg/mL)**	$1/q_e$ **(g cotton/mg)**	$1/C_e$ **(mL/mg)**	**ln** q_e	**ln** C_e
85	0.5045	2.38	0.0610	0.4200	16.39	0.87	-2.80
134	0.5023	3.56	0.0984	0.2810	10.16	1.27	-2.32
182	0.5078	4.43	0.1368	0.2258	7.31	1.49	-1.99
232	0.5059	5.48	0.1763	0.1825	5.67	1.70	-1.74
328	0.5002	6.75	0.2602	0.1482	3.84	1.91	-1.35
430	0.5017	8.65	0.3436	0.1156	2.91	2.16	-1.07
630	0.5091	11.2	0.5164	0.0893	1.94	2.42	-0.66
819	0.5014	13.6	0.6821	0.0735	1.47	2.61	-0.38

Table 4.26 Data for the adsorption isotherm of lac dye on cotton in the presence of 0.5 M sodium chloride without pH control at the initial dye range 85-819 mg/L, MLR 1:100 and 30 ^{o}C; pH before and after dyeing = 5.57 and 6.32 respectively

Initial conc. (mg/L)	**Weight cotton (g)**	q_e **(mg/ g cotton)**	C_e **(mg/mL)**	$1/q_e$ **(g cotton/mg)**	$1/C_e$ **(mL/mg)**	**ln** q_e	**ln** C_e
85	0.5051	1.59	0.0689	0.6275	14.507	0.47	-2.67
134	0.5031	2.35	0.1105	0.4259	9.049	0.85	-2.20
182	0.5025	3.14	0.1502	0.3185	6.658	1.14	-1.90
232	0.5031	3.65	0.1950	0.2738	5.128	1.30	-1.63
328	0.5005	4.94	0.2782	0.2023	3.595	1.60	-1.28
430	0.5063	6.60	0.3636	0.1516	2.750	1.89	-1.01
630	0.5044	6.82	0.5616	0.1466	1.781	1.92	-0.58
819	0.5082	9.68	0.7202	0.1033	1.389	2.27	-0.33

The graph is plotted in the form of the dye adsorbed per gram of cotton, q_e, against the concentration of dye remaining in solution, C_e, with the Langmuir equation as shown in Figure 4.18. As seen from Figures 4.19 and 4.20, the linear forms of the Langmuir and Freundlich isotherms fitted quite well with the experimental data with high correlation coefficients ($R^2 > 0.99$). Parameters of the Langmuir and Freundlich isotherms together with the correlation coefficients in this study were computed in Table 4.27. It was found that the adsorption capacity (Q) values decreased with increasing temperature. The same trend was also observed from the Freundlich parameter that the Q_f values decreased with an increasing in temperature. These

results clearly indicated that the adsorption of lac dye on cotton in the presence of 0.5 M sodium chloride without pH control is an exothermic process.

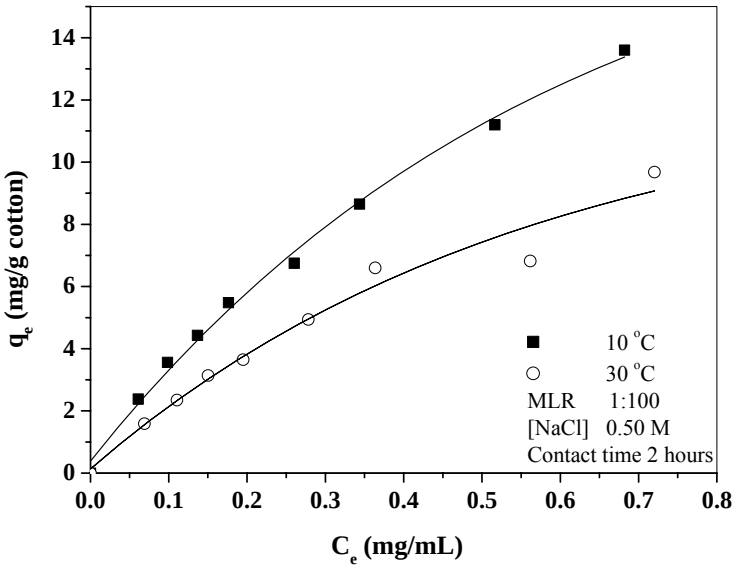


Figure 4.18 Adsorption isotherm of lac dye on cotton in the presence of 0.5 M sodium chloride without pH control.

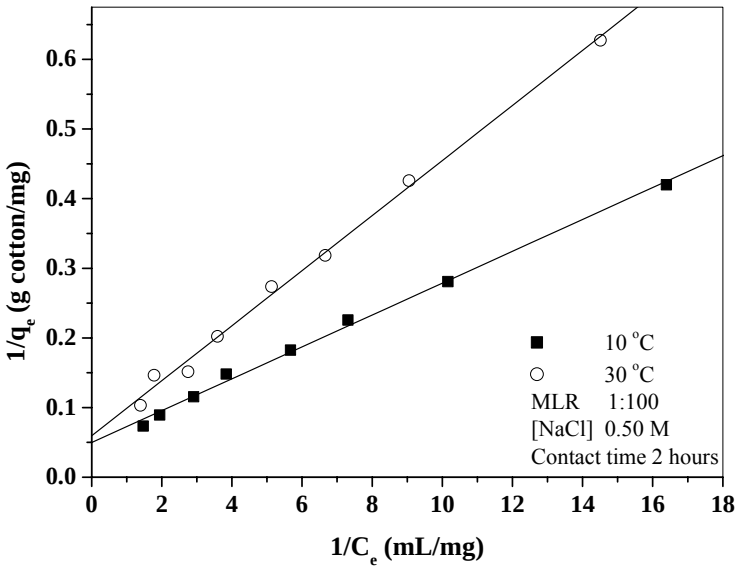


Figure 4.19 A plot of $1/q_e$ against $1/C_e$ for the adsorption of lac dye on cotton in the presence of 0.5 M sodium chloride without pH control.

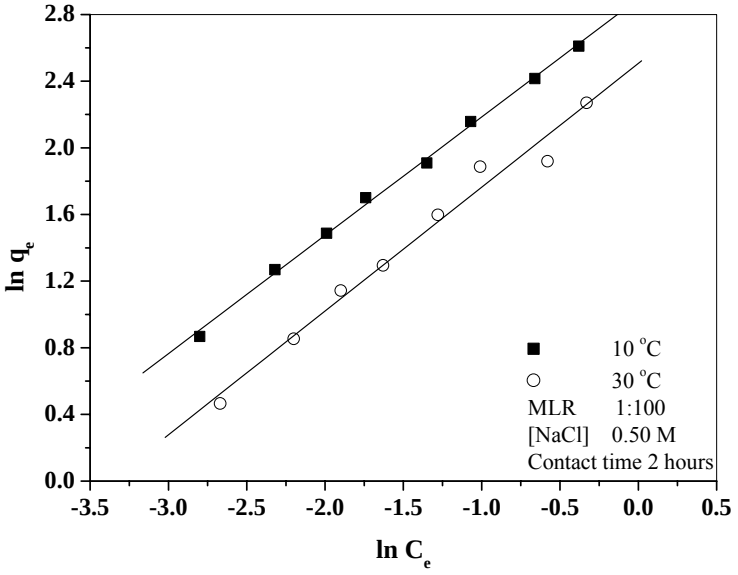


Figure 4.20 Freundlich adsorption isotherm of lac dye on cotton in the presence of 0.5 M sodium chloride without pH control**.**

Table 4.27 Langmuir and Freundlich isotherm constants of adsorption of lac dye on cotton in the presence of 0.5 M sodium chloride without pH control at 10 and 30 ^{o}C, MLR 1:100 and initial dye concentration range 85-819 mg/L.

Temp (^{o}C)	Langmuir			Freundlich		
	Q (mg/g cotton)	b (mL/mg)	R^2	Q_f (mg/g cotton)	n	R^2
10	20.04	2.18	0.9982	18.08	1.41	0.9989
30	16.78	1.51	0.9978	12.26	1.35	0.9903

The thermodynamic parameters for the adsorption of lac dye on cotton in the presence of 0.5 M sodium chloride without pH control at 10 and 30 ^{o}C were also calculated using equations (4.3), (4.4) and (4.6) respectively. The results are reported in Table 4.28. The small negative value of enthalpy change (ΔH^{o}) suggests the adsorption to be physical (Jain *et al*., 2003). The negative values of free energy (ΔG^{o}) show the spontaneous nature of adsorption process.

Table 4.28 Thermodynamic parameters for the adsorption of lac dye on cotton in the presence of 0.5 M sodium chloride without pH control at 10 and 30 ^{o}C, MLR 1:100 and initial dye concentration range 85-819 mg/L.

Temp (^{o}C)	b (mL/mg)	ΔG^{o} (kJ/mol)	ΔH^{o} (kJ/mol)	ΔS^{o} (J/mol K)
10	2.18	-1.83	-13.09	-39.79
30	1.51	-1.04		-39.77

4.3.4.4 Adsorption isotherm of lac dye on pretreated cotton with chitosan at pH 3.0

As mentioned above in section 4.1, lac dye has a small affinity for cotton because of the absence of cationic sites on cotton for attachment. For this reason, mordants such as alum, ferrous sulphate and tin have been used for lac dyeing onto cotton thus improving the fastness properties (Moeyes, 1993). However, the wastewater containing these mordants may affect the environment and public health. To avoid this hazardous problem, the pretreatment of cotton with chitosan, a naturally occurring polymer containing amino groups, is an alternative way to introduce cationic sites on cotton. According to Saxena *et al*. (1997), the pretreatment of cotton with chitosan enhanced the affinity of lac dye on cotton. However, the adsorption isotherm was not reported in their study. Therefore, the adsorption of lac dye on pretreated cotton with chitosan at pH 3.0 was studied in this section in order to obtain the thermodynamic parameters. The cotton yarn was treated with 0.3% and 0.6% (v/v) aqueous solutions of chitosan. It was found that the pretreated cotton with a 0.6% (v/v) aqueous solution of chitosan provided an unsmooth coating which resulted in an uneven distribution of dye adsorbed on cotton yarn. Therefore, the cotton treated with a 0.3% aqueous (v/v) solution of chitosan was chosen to study the adsorption isotherm of lac dye. The equilibrium data at 10, 30 and 60 ^{o}C are given in Tables 4.29-4.31.

Table 4.29 Data for the adsorption isotherm of lac dye on pretreated cotton with chitosan; dyeing at the initial dye concentration range 29-469 mg/L, MLR 1:100, pH 3.0 and 10 ^{o}C

Initial conc. (mg/L)	**Weight cotton (g)**	$\mathbf{A_o}$	$\mathbf{A_{t = 3\ hrs}}$	q_e (mg/g cotton)	C_e (mg/mL)	C_e/q_e (g cotton/mL)
29	0.5040	0.1233	0.0214	2.38	0.0052	2.18×10^{-3}
57	0.5072	0.2370	0.0446	4.52	0.0108	2.39×10^{-3}
112	0.5061	0.4670	0.1439	7.64	0.0348	4.55×10^{-3}
170	0.5025	0.7048	0.2671	10.45	0.0645	6.17×10^{-3}
210	0.5014	0.8729	0.3741	11.95	0.0904	7.56×10^{-3}
263	0.5045	1.0900	0.5380	13.15	0.1300	9.88×10^{-3}
362	0.5040	1.5029	0.8944	14.52	0.2160	1.49×10^{-2}
469	0.5034	1.9442	1.2949	15.51	0.3128	2.02×10^{-2}

Table 4.30 Data for the adsorption isotherm of lac dye on pretreated cotton with chitosan; dyeing at the initial dye concentration range 29-469 mg/L, MLR 1:100, pH 3.0 and 30 ^{o}C

Initial conc. (mg/L)	**Weight cotton (g)**	$\mathbf{A_o}$	$\mathbf{A_{t = 3\ hrs}}$	q_e (mg/g cotton)	C_e (mg/mL)	C_e/q_e (g cotton/mL)
29	0.5021	0.1233	0.0266	2.26	0.0064	2.84×10^{-3}
57	0.5034	0.2370	0.0803	3.69	0.0194	5.25×10^{-3}
112	0.5016	0.4670	0.2272	5.71	0.0549	9.62×10^{-3}
170	0.5053	0.7048	0.3625	8.12	0.0876	1.08×10^{-2}
210	0.5036	0.8729	0.4800	9.36	0.1159	1.24×10^{-2}
263	0.5012	1.0900	0.6312	10.99	0.1525	1.39×10^{-2}
362	0.5040	1.5029	1.0021	11.93	0.2421	2.03×10^{-2}
469	0.5078	1.9442	1.4181	12.45	0.3425	2.75×10^{-2}

Table 4.31 Data for the adsorption isotherm of lac dye on pretreated cotton with chitosan; dyeing at the initial dye concentration range 112-469 mg/L, MLR 1:100, pH 3.0 and 60 ^{o}C

Initial conc. (mg/L)	**Weight cotton (g)**	$\mathbf{A_o}$	$\mathbf{A_{t = 3\ hrs}}$	q_e (mg/g cotton)	C_e (mg/mL)	C_e/q_e (g cotton/mL)
112	0.5048	0.4670	0.2944	4.06	0.0711	1.75×10^{-2}
170	0.5061	0.7048	0.4410	6.23	0.1065	1.71×10^{-2}
210	0.5044	0.8729	0.6311	5.72	0.1524	2.66×10^{-2}
263	0.5043	1.0900	0.8020	6.83	0.1937	2.84×10^{-2}
362	0.5058	1.5029	1.1701	7.88	0.2826	3.59×10^{-2}
469	0.5063	1.9442	1.5844	8.52	0.3827	4.49×10^{-2}

The Langmuir and Freundlich isotherm models were examined in this study to describe the adsorption equilibrium. Figure 4.21 shows the adsorption isotherm of lac dye on pretreated cotton. The linear plots of C_e/q_e versus C_e following equation (3.12) show that the adsorption yielded good fits with the Langmuir isotherm model (Figure 4.22).

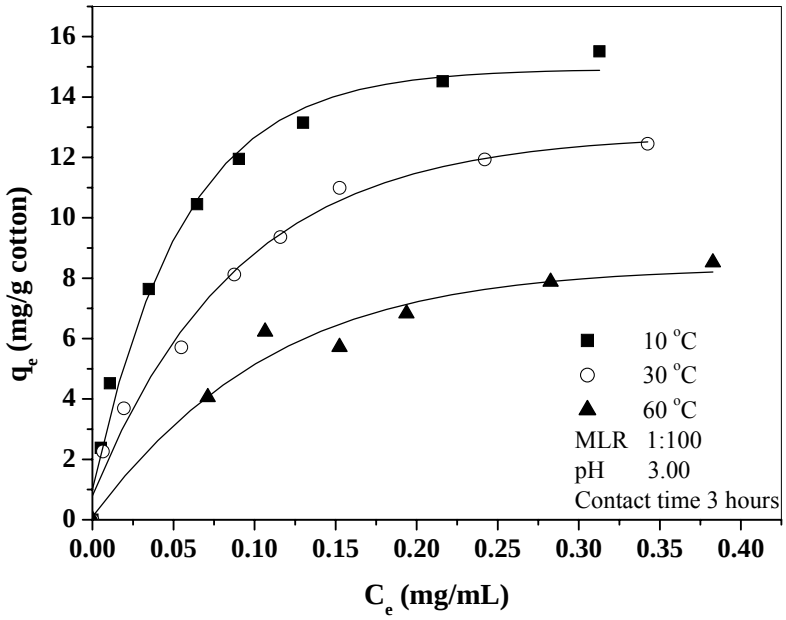


Figure 4.21 Adsorption isotherm of lac dye on pretreated cotton with chitosan in the initial dye concentration range 29-469 mg/L.

The correlation coefficients (R^2) were higher than 0.98. Adsorption capacity (Q) and the Langmuir constant (b) were determined from the slope and intercept of this plot as listed in Table 4.32. It was found that the adsorption capacity decreased with an increasing in the temperature, indicating an exothermic process. Based on the correlation coefficient (R^2) shown in Table 4.32, the equilibrium data could not be described by the isotherm of Freundlich (Figure 4.23).

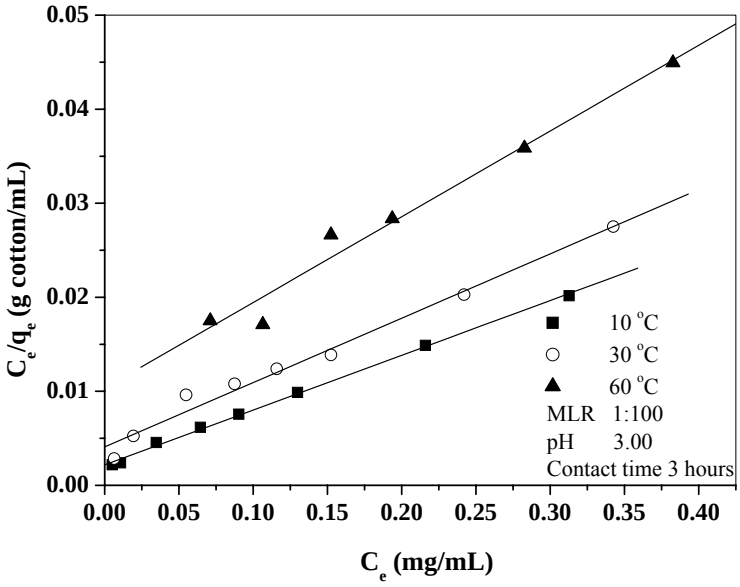


Figure 4.22 A plot of C_e/q_e against C_e for the adsorption of lac dye on pretreated cotton with chitosan in the initial dye concentration range 29-469 mg/L.

Table 4.32 Langmuir and Freundlich isotherm constants of the adsorption of lac dye on pretreated cotton with chitosan at different temperatures; dyeing at the initial dye concentration range 29-469 mg/L, MLR 1:100 and pH 3.0

Temp (°C)	Langmuir			Freundlich		
	Q (mg/g cotton)	b (mL/mg)	R^2	Q_f (mg/g cotton)	n	R^2
10	17.16	26.73	0.9990	31.38	2.23	0.9770
30	14.61	16.78	0.9918	23.24	2.18	0.9910
60	10.97	8.82	0.9868	12.89	2.51	0.9366

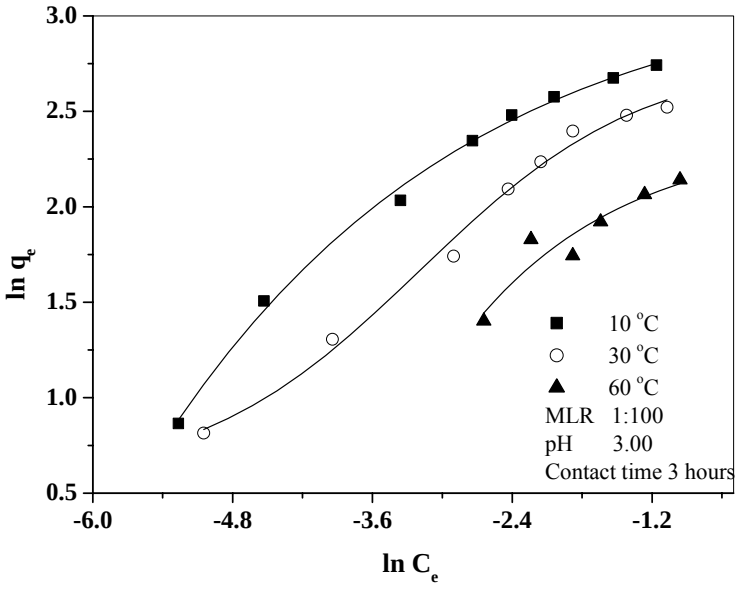


Figure 4.23 Freundlich isotherm for the adsorption of lac dye on pretreated cotton with chitosan in the initial dye concentration range 29-469 mg/L.

To obtain the thermodynamic parameters, equation (4.3) was used to calculate the free energy (ΔG^o). According to equation (4.5), a linearized plot of ln (b) versus $1/T$ is obtained as shown in Figure 4.24. Enthalpy (ΔH^o) and entropy (ΔS^o) of the adsorption were calculated from the slope and intercept of this line, respectively. The calculated results are reported in Table 4.33. Based on these results, it can be concluded that the adsorption of lac dye on pretreated cotton with chitosan at pH 3.0 is a spontaneous and exothermic process.

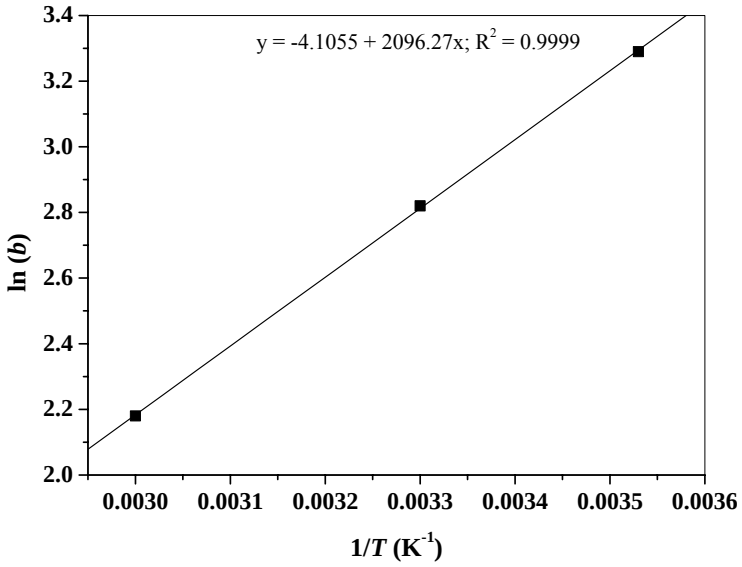


Figure 4.24 A plot of ln (*b*) against 1/*T* for the adsorption of lac dye on pretreated cotton with chitosan in the initial dye concentration range 29-469 mg/L.

Table 4.33 Thermodynamic parameters for the adsorption of lac dye on pretreated cotton with chitosan at different temperatures; dyeing at the initial dye concentration range 29-469 mg/L, MLR 1:100 and pH 3.0

Temp (^{o}C)	***b*** **(mL/mg)**	**ΔG^o (kJ/mol)**	**ΔH^o (kJ/mol)**	**ΔS^o (J/mol K)**	***R^2***
10	26.73	-7.73			
30	16.78	-7.10	-17.43	-34.13	0.9999
60	8.82	-6.03			

A schematic diagram of cotton-chitosan-lac dye interaction is presented in Figure 4.25. The chitosan contains amine groups, $–NH_2$, which are easily protonated to form the protonated amino ($-NH_3^+$) cations in acidic solution (Majeti and Kumar, 2000). At a pH lower than 4, the carboxylic acid and hydroxyl groups on cellulose would not be ionized. After pretreatment of the cotton with chitosan under acidic conditions, the protonated amino ($-NH_3^+$) groups of chitosan can interact with the surface of the cellulose based on hydrogen bonding and ion-dipole interactions. The pretreated cotton was then dyed with lac dye under acidic conditions. Lac dye molecules, containing carboxylic acid (–COOH) groups, hydroxyl (–OH) groups and amide (–NHCO–) groups in laccaic acid A (major component), could interact with pretreated cotton via hydrogen bonding and ion-dipole interactions. In this way chitosan could act as an organic mordant to enhance the uptake of lac dye on the cellulose surface resulting in lac dye sorption on the cotton. Therefore, the calculated enthalpy change was higher than that from the lac dyeing in the presence of 0.5 M NaCl without pH control and lac dyeing on cotton at pH 3.0 respectively. The results are given in Table 4.34.

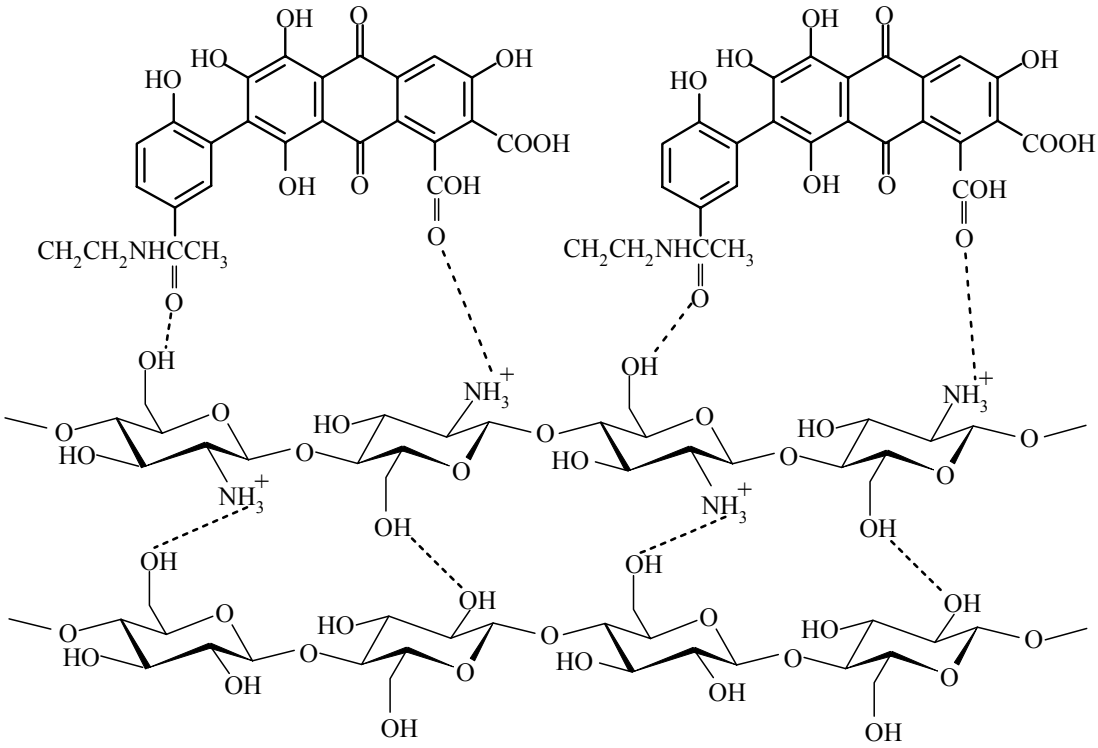


Figure 4.25 Schematic diagram of cotton-chitosan-lac dye interaction.

4.3.4.5 Comparison of thermodynamic parameters between the adsorption of lac dye on untreated and pretreated cotton with chitosan

Under the same condition, the amount of the dye adsorbed on pretreated cotton with a 0.3% aqueous (v/v) aqueous solution of chitosan was higher than that on the untreated cotton as shown in Figure 4.26. This may be due to the protonated amino group of chitosan forming interaction with both cellulose and lac dye, and acting like a binder between the two which results in higher dye adsorption. In addition, the amount of the dye adsorbed on pretreated cotton with chitosan was also higher than that on the untreated cotton in the presence of 0.5 M NaCl without

pH control. It showed that chitosan plays an important role compared to sodium chloride salt in the lac dyeing onto cotton.

Under the same dyeing conditions, the thermodynamic parameters of the adsorption of lac dye on untreated cotton with chitosan were compared with that of the adsorption of lac dye on pretreated cotton with chitosan at pH 3.0 as shown in Table 4.34. It was found that the free energy in both is a spontaneous process. The enthalpy obtained from the adsorption of lac dye on pretreated cotton with chitosan was higher than that of the untreated cotton. This supported that the dye molecules can easily attract to chitosan which interacts with the surface of cotton. Therefore, the pretreated cotton with chitosan enhanced the adsorption capacity in lac dye on cotton.

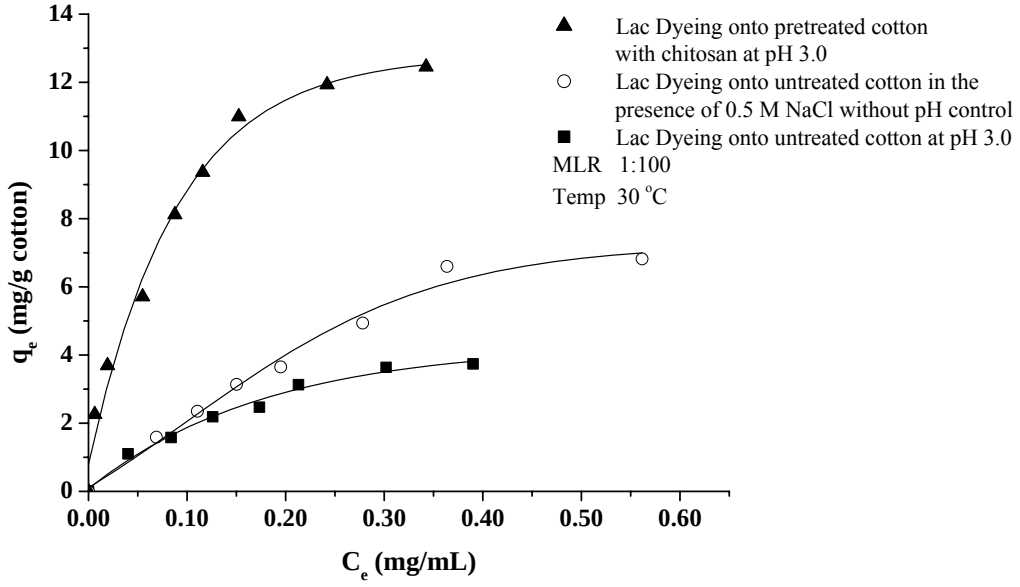


Figure 4.26 Comparison of the adsorption isotherm of lac dye on pretreated and untreated cotton with chitosan at pH 3.0 and lac dye on cotton in the presence of 0.5 M NaCl without pH control.

Table 4.34 Thermodynamic parameters for the adsorption of lac dye on untreated and pretreated cotton with chitosan at pH 3.0, and lac dye on cotton in the presence of 0.5 M NaCl without pH control

Temp (^{o}**C**)	***b*** **(mL/mg)**	**ΔG^{o} (kJ/mol)**	**ΔH^{o} (kJ/mol)**	**ΔS^{o} (J/mol K)**	***R*^{2}**
Lac dyeing onto untreated cotton with chitosan at pH 3.0					
10	6.76	-4.50			
30	5.98	-4.51	-4.88	-1.31	0.9986
60	4.93	-4.42			
Lac dyeing onto pretreated cotton with chitosan at pH 3.0					
10	26.73	-7.73			
30	16.78	-7.10	-17.43	-34.13	0.9999
60	8.82	-6.03			
Lac dyeing onto untreated cotton in the presence of 0.5 M NaCl without pH control					
10	2.18	-1.83	-13.09	-39.79	
30	1.51	-1.04		-39.77	

4.3.4.6 Adsorption and desorption study of lac dye on untreated and pretreated cotton with chitosan at pH 3.0 and on cotton in the presence of 0.5 M NaCl without pH control

The adsorption and desorption data of lac dye on pretreated and untreated cotton with chitosan are given in Tables 4.35 and 4.36 respectively. Also the adsorption and desorption data of lac dye on cotton in the presence of 0.5 M NaCl without pH control is given in Table 4.37. As seen from Figure 4.27, cotton pretreated with chitosan showed a higher dye uptake than the untreated cotton. Also, the cotton dyeing of the pretreated cotton with chitosan enhanced dye uptake compared with cotton dyed in the presence of sodium chloride. This indicated that the modification of cotton by pretreatment with chitosan can create an affinity between cotton and lac dye (Saxena *et al*., 1997). After 180 minutes, the dye bath was replaced with deionized water and desorption of lac was observed. The amount of lac dye which desorbed from the cotton in all cases was shown in Figure 4.27. At 420 minutes, the amount of the dye adsorbed on pretreated and untreated cotton with chitosan at pH 3.0 and in the presence of NaCl was 8.8, 2.4 and 1.1 mg/g cotton respectively. It was found that lac dye can desorbed easily from the cotton which was dyed in the presence of NaCl and at pH 3.0 without the pretreatment with chitosan. This data supported the belief that the pretreatment of cotton with chitosan in the lac dyeing process increased the amount of dye adsorbed onto the cotton and also decreased the dye desorption from cotton.

Table 4.35 The adsorption and desorption data of lac dye on pretreated cotton with chitosan at pH 3.0, MLR 1:100 and 30 ^{o}C

Time (min)	**Weight cotton** (g)	A_o	A_t	q_t (mg/g cotton)	**Time for desorption** (min)	q_{de} (mg/g cotton)	q_e- q_{de} (mg/g cotton)
0	-	1.9619	-	0.00	181	6.2	13.4
1	0.5062	1.9619	1.4897	11.20	182	7.3	12.4
2	0.5018	1.9619	1.4391	12.52	185	8.6	11.1
3	0.5071	1.9619	1.4157	12.94	190	9.2	10.4
4	0.5035	1.9619	1.3604	14.36	200	10.2	9.5
5	0.5063	1.9619	1.3600	14.29	210	9.9	9.8
6	0.5058	1.9619	1.3546	14.43	240	10.6	9.0
7	0.5041	1.9619	1.2884	16.07	300	10.4	9.3
8	0.5096	1.9619	1.3211	15.12	360	10.9	8.8
9	0.5088	1.9619	1.3065	15.49	420	10.9	8.8
10	0.5088	1.9619	1.2898	15.89			
12	0.5090	1.9619	1.2787	16.14			
15	0.5066	1.9619	1.2568	16.74			
20	0.5040	1.9619	1.2653	16.63			
30	0.5093	1.9619	1.2395	17.06			
60	0.5084	1.9619	1.2006	18.02			
90	0.5043	1.9619	1.1525	19.32			
120	0.5026	1.9619	1.1299	19.93			
180	0.5029	1.9619	1.1400	19.67			

Table 4.36 The adsorption and desorption data of lac dye on untreated cotton with chitosan at pH 3.0, MLR 1:100 and 30 ^{o}C

Time (min)	**Weight cotton** (g)	A_o	A_t	q_t (mg/g cotton)	**Time for desorption** (min)	q_{de} (mg/g cotton)	q_e- q_{de} (mg/g cotton)
0	-	1.9619	-	0.00	181	1.1	3.3
1	0.5038	1.9619	1.9104	1.17	182	1.4	3.1
3	0.5062	1.9619	1.8872	1.72	185	1.7	2.7
4	0.5055	1.9619	1.8746	2.02	190	1.9	2.6
5	0.5047	1.9619	1.8736	2.05	200	2.0	2.5
6	0.5021	1.9619	1.8551	2.50	210	2.1	2.4
7	0.5020	1.9619	1.8510	2.60	240	2.0	2.4
8	0.5056	1.9619	1.8562	2.46	300	2.0	2.4
9	0.5020	1.9619	1.8496	2.64	360	2.1	2.3
10	0.5090	1.9619	1.8427	2.76	420	2.0	2.4
12	0.5028	1.9619	1.8353	2.97			
15	0.5086	1.9619	1.8344	2.96			
20	0.5083	1.9619	1.8211	3.28			
30	0.5044	1.9619	1.8058	3.67			
60	0.5085	1.9619	1.7772	4.32			
90	0.5051	1.9619	1.7734	4.44			
120	0.5021	1.9619	1.7753	4.42			
180	0.5030	1.9619	1.7735	4.46			

Table 4.37 The adsorption and desorption data of lac dye on cotton in the presence of 0.5 M NaCl without pH control, MLR 1:100 and 30 ^{o}C

Time (min)	**Weight cotton** (g)	A_o	A_t	q_t (mg/g cotton)	**Time for desorption** (min)	q_{de} (mg/g cotton)	q_e- q_{de} (mg/g cotton)
0	-	1.8822		0.00	181	3.7	4.2
1	0.5031	1.8822	1.7662	3.74	182	4.5	3.4
2	0.5021	1.8822	1.7701	3.65	185	7.1	0.8
3	0.5090	1.8822	1.7334	4.50	190	7.5	0.4
4	0.5056	1.8822	1.7115	5.08	200	7.5	0.4
5	0.5077	1.8822	1.7278	4.65	210	6.3	1.6
6	0.5029	1.8822	1.6981	5.44	240	6.4	1.5
7	0.5055	1.8822	1.6989	5.39	360	6.4	1.4
8	0.5052	1.8822	1.6622	6.30	420	6.8	1.1
9	0.5023	1.8822	1.6767	5.98			
10	0.5040	1.8822	1.6611	6.34			
12	0.5052	1.8822	1.6318	7.05			
15	0.5014	1.8822	1.6228	7.33			
20	0.5070	1.8822	1.6122	7.51			
30	0.5096	1.8822	1.6220	7.23			
60	0.5074	1.8822	1.6369	6.90			
90	0.5010	1.8822	1.6112	7.62			
180	0.5021	1.8822	1.6008	7.87			

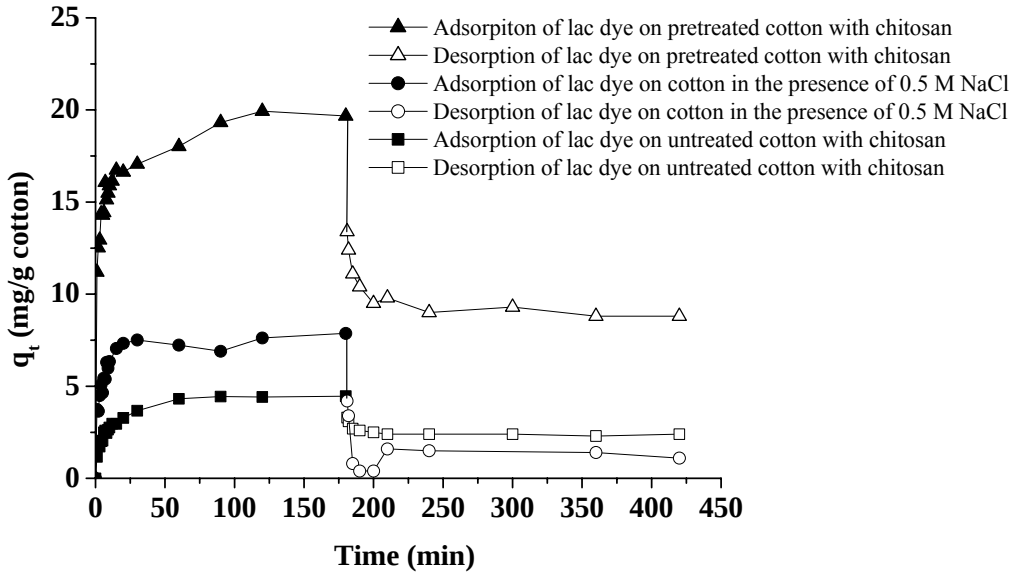


Figure 4.27 Adsorption and desorption of lac dye on cotton.

4.4 Conclusions

This study investigated the adsorption kinetics and thermodynamics of lac dye on cotton. The adsorption capacities are significantly affected by pH, the initial dye concentration, the material to liquor ratio (MLR) and temperature. It was found that dye uptake increased with decreasing pH. Also, the dye uptake increased at higher initial concentration of the lac dye and was influenced by the material to liquor ratio (MLR). In addition, the initial dye adsorption rates (h_i) onto cotton before equilibrium time increased at higher dyeing temperatures. Therefore, the adsorption kinetics were investigated using the optimal conditions of a pH of 3.0, a material to liquor ratio (MLR) of 1:100, an initial dye concentration of 480±10 mg/L and 120 minutes contact time. The adsorption kinetics of lac dye on cotton was found to follow the pseudo second-order kinetic model. The activation energy for the adsorption of lac

dye on cotton was evaluated using the pseudo second-order rate constants. Batch isotherm studies showed that the adsorption of lac dye on cotton was described by the Langmuir and Freundlich isotherms. The enthalpy (ΔH^o) for the adsorption of lac dye on cotton at pH 3.0 was found to be -4.88 kJ/mol indicating that the process is exothermic. The small negative value of ΔH^o suggested the adsorption to be physical.

Due to the low affinity between lac dye and cotton, the effect of sodium chloride, and pretreatment of cotton with chitosan, on the adsorption of lac dyeing was studied. These results were compared with cotton not treated with chitosan. The results revealed that an increase in the sodium chloride concentration over the range 4.31×10^{-3} - 6.84×10^{-1} M led to an increase in the dye adsorbed onto the cotton and remained constant in the concentration range 10.3×10^{-1} - 17.1×10^{-1} M when cotton fibres were dyed with lac dye solution without pH control. It is believed that the sodium ion (Na^+) can distribute between the external solution and internal solution so that the negative charge on the cellulose surface is neutralized or shielded, allowing the lac dye molecules to be adsorbed onto the cotton (Horrocks and Anand, 2000; Yang, 1998; Stana-Klenschek and Ribisch, 1998). The adsorption isotherm of lac dye on cotton in the presence of 0.5 M NaCl agreed well with both the Langmuir and Freundlich isotherms with an enthalpy (ΔH^o) of -13.09 kJ/mol. However, sodium chloride has no effect on the adsorption of lac dye on cotton at pH 2.5, 3.0 and 3.5 respectively. It indicated that hydrogen ions (H^+) play a more important role than sodium ions (Na^+). The pretreatment of cotton with a 0.3% aqueous (v/v) solution of chitosan showed a prominent enhancement of dye uptake onto the cotton and also a decrease in the dye desorbed from cotton compared with the results in the absence of chitosan or on lac dyeing in the presence of NaCl. The experimental data for lac

dyeing on cotton pretreated with chitosan fitted very well to the Langmuir isotherm with an enthalpy (ΔH^{o}) of -17.43 kJ/mol. It was concluded that the pretreatment of cotton with chitosan is an alternative way to create cationic sites on the cotton and subsequently increased the affinity between lac dye and cotton.

CHAPTER V

AN ABSORPTION SPECTROSCOPIC INVESTIGATION OF THE INTERACTION OF LAC DYES WITH METAL IONS

5.1 INTRODUCTION

Nowadays, there is a renewed interest in the dyeing of textile fibres with natural dyes because of the desire to attain high compatibility with the environment, lower toxicity and to prevent allergic reactions (Cristea, Bareau and Vilarem, 2003). However, problems in dyeing with natural dyes are related to the low exhaustion of the dyes and to the fastness of the dyed fabrics. Attempts to overcome these problems have been focused on the application of metallic salts as mordants, which are traditionally used to improve the fastness properties of exhaustion and to develop different shades with the same dye (Bechtold, Turcanu, Ganglberger and Geissler, 2003). A mordant is an element which aids the chemical reaction that takes place between the dye and the fibre so that the dye is adsorbed. There are different methods of applying mordants in the dyeing process such as pre-mordanting before dyeing, stuffing (mordanting and dyeing at the same time), after-mordanting, and a combination of pre-mordanting and after-mordanting (Moeyes, 1993). There are numerous studies on the use of metals as mordants in dyeing process, such as

Kongdee and Bechtold (2004) who used the $Fe(OH)_3$ for the dyeing of cellulosic fibres under alkaline solution. Five different mordanting metal ions, Cr(III), Al(III), Fe(II), Cu(II), and Sn(II) have been applied to dye baths in wool dyeing (Guzel and Akgerman, 2000). Raisanen, Nousiainen and Hynninen (2001) presented results using the pure, natural anthraquinones emodin and dermocybin on wool and polyamide using various mordants, e.g., alum, potassium aluminum sulphate, potassium dichromate, cobaltous sulphate, and ferrous sulphate.

In view of the use of metal ions as mordants, their interaction with dyes is important. The interaction of lac dye with metal ions has not been reported yet, however the interaction of other dyes with metal ions have been published. For instance, the visible absorption spectrum of stilbazolium merocyanine in the presence of Cu(II), Co(II) and Fe(III) ions has been investigated using UV-Visible spectroscopy (Cegielski, Niedbalska and Manikowski, 2001). It was found that both the intensity of the maximum absorption and its position were related to changes in the metal ion concentrations. The complexation of 5-hydroxyflavone with Al(III) was investigated by using UV-Visible and Raman spectroscopies (Cornard and Merlin, 2001). In addition, the structure stability and molar absorptivity of the complex formed between $AlCl_3$ and 5,7-dihydroxy-flavone in methanol were investigated using UV-Visible spectroscopy and AM1 method (Castro and Blanco, 2004). As part of the dyeing process with lac dye on cotton and silk, alum, potassium dichromate, stannous chloride and copper sulfate have been used as mordants to improve colour fastness (Nakamura and Matumoto, 1980; Moeyes, 1993; Saxena *et al*., 1997; Patra, 1998; Rastogi *et al*., 2000). While metal ion mordants have also been used by others in dyeing with lac dyes, there have not been any reports on specific spectroscopic

studies involving these components. In an effort to gain a greater understanding of the action of these mordants, and with a view ultimately to improving the reliability and quality of the dyeing process, a systematic study of the interaction of lac dyes and laccaic acids with selected metal ions will be undertaken. In particular, metal ion and pH effects on the visible absorption spectra of Thai lac dye extracted from stick lac from the Rain tree in northeast Thailand will be investigated. Also, comparative studies will be done with commercial lac dye (Wako Company) and laccaic acids A and B in order to ascertain if the Thai lac dye had any distinctively different characteristics. The results of this study are reported in this chapter.

5.2 Experimental

5.2.1 Chemicals

Laccaic acid A [15979-35-8], laccaic acid B [17249-00-2], and lac dye [124-04012] were purchased from the Wako Company (Osaka, Japan). For simplicity, the last compound is referred to as Wako lac dye. Analytical grade metal ion salts, $Ni(NO_3)_2 \cdot 6H_2O$ and $KAl(SO_4)_2 \cdot 12H_2O$ (alum) were purchased from the Merck Co., Ltd.

5.2.2 Instruments

(a) A Cary 1E UV-Visible spectrophotometer was employed for absorbance measurements using quartz cells of path length 1 cm.

(b) A pH meter (Mettler Delta 320, UK) was used to measure the pH values of the lac dye solutions.

(c) Freeze dyer (Heto FD3 model S/N 492497-B, Cat No. 837107, Denmark) was used to dry the crude extract.

(d) Rotary evaporator (Büchi Rotavaor R-114) was employed to remove the water from the crude extract.

5.2.3 Experimental methods

5.2.3.1 Extraction of lac dye

Stick lac (500.48 g) collected from Rain trees in northeast Thailand (Nakhon Ratchasima Province) was powdered in a grinding mill and finely ground (18 mesh). The powdered material was extracted with deionized water (1.5 L) at 60 ^{o}C for 1 hour. The aqueous solution was filtered and then concentrated under reduced

pressure (rotary evaporator) to give a crude lac dye extract (38.33 g), which was then used without further purification. This extract is referred to as Thai lac dye.

5.2.3.2 Measurement of pH and spectra

The influence of pH on the visible absorption spectra of Wako lac dye and Thai lac dye was determined using 3% (v/v) acetic acid solution in the pH range 2.5-4.5, and 0.5 M sodium hydroxide solution in the pH range 5.0-11.0. The pH values were measured using a pH meter (Mettler Delta 320, UK). The UV-Visible spectra were recorded using a Cary 1E UV-Visible spectrophotometer. This instrument was used for all UV-Visible absorption spectra in this work.

5.2.3.3 Metal ion effects

(a) Effect of metal ion concentration on laccaic acid A and laccaic acid B visible absorption spectra in deionized water

The final concentration of laccaic acids A and B in the measured aqueous solutions (deionized water) was 9.31×10^{-5} and 1.05×10^{-4} M respectively. The final concentrations of metal ions in the laccaic acids A and B solution were varied as follows: 5.0×10^{-5}, 2.0×10^{-4}, 5.0×10^{-4}, 2.0×10^{-3}, 5.0×10^{-3}, 7.0×10^{-3}, 1.0×10^{-2}, 1.5×10^{-2}, 2.0×10^{-2}, 2.5×10^{-2}, 3.0×10^{-2}, 3.5×10^{-2}, and 4.0×10^{-2} M. The pH of the final solution in each case was measured using a pH meter.

(b) Alum solution studies on the visible absorption spectra of Wako lac dye and Thai lac dye

Wako and Thai Lac dye solutions were freshly prepared prior to each determination. Wako lac dye (1.0364 g) was partially dissolved in deionized water, the mixture filtered, and the filtrate diluted to 500 mL with deionized water in a 500 mL volumetric flask. The Thai lac dye solution was prepared by treating the dye

(0.5024 g) with deionized water (400 mL). This solution was filtered and the filtrate diluted to 500 mL with deionized water in a 500 mL volumetric flask. Into two series of twelve 10 mL volumetric flasks were added 0.50 mL of each lac dye solution. To the flasks, was added successively, 0.10, 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 4.00, 5.00, 6.00, 7.00, and 8.00 mL of aqueous $5x10^{-2}$ M $KAl(SO_4)_2 \cdot 12H_2O$ (alum). Each flask was then diluted to the mark with deionized water and the solutions allowed to stand for 30 min. After complex formation was complete, the absorption spectra and the changes in absorbance at 537 nm were recorded on a Cary 1E UV-Vis spectrophotometer against deionized water as the blank, and using quartz cells. The final concentrations of the alum solution in this experiment were as follows: $5.0x10^{-4}$, $2.5x10^{-3}$, $5.0x10^{-3}$, $7.5x10^{-3}$, $1.0x10^{-2}$, $1.25x10^{-2}$, $1.5x10^{-2}$, $2.0x10^{-2}$, $2.5x10^{-2}$, $3.0x10^{-2}$, $3.5x10^{-2}$, and $4.0x\ 10^{-2}$ M.

(c) Nickel (II) ion solution studies on the visible absorption spectra of Wako lac dye and Thai lac dye

Lac dye solutions were prepared as noted in section 2.3.2.2. Into each series of twelve 10 mL volumetric flasks was added 0.50 mL of lac dye solution. To the flasks, was then added consecutively, 0.10, 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 4.00, 5.00, 6.00, 7.00, and 8.00 mL of aqueous $5x10^{-2}$ M $Ni(NO_3)_2 \cdot 6H_2O$. Each flask was diluted to the mark with deionized water and the solutions then kept at room temperature for 30 min. After complex formation was complete, the absorption spectra and the changes in absorbance at 562 nm were recorded on a Cary 1E UV-Vis spectrophotometer against deionized water as the blank and using quartz cells. The final concentrations of nickel (II) solution in this experiment were as follows :

$5.0x10^{-4}$, $2.5x10^{-3}$, $5.0x10^{-3}$, $7.5x10^{-3}$, $1.0x10^{-2}$, $1.25x10^{-2}$, $1.5x10^{-2}$, $2.0x10^{-2}$, $2.5x10^{-2}$, $3.0x10^{-2}$, $3.5x10^{-2}$, and $4.0x10^{-2}$ M.

5.2.3.4 Determination of the mole ratio for the nickel (II) ion – laccaic acid A complex

The continuous variation (Job's) method was used to determine stoichiometry (Beck, 1970). A series of aqueous solutions were prepared by mixing $5x10^{-4}$ M laccaic acid A and $5x10^{-4}$ M $Ni(NO_3)_2 \cdot 6H_2O$ and diluting to 5.00 ml with ethanol. The pH of each solution was measured, and the pH range observed was 2.8-4.2. The mixtures were allowed to stand at room temperature for at least 30 min and the changes in absorbance at λ_{max} of the longest wavelength absorption band were then measured.

5.2.3.5 Computational studies

Computational studies on the nickel (II) ion - laccaic acid A complex were carried out on a Silicon Graphics Fuel processor running at 600 MHz with 1536 Mbytes of random access memory using the Spartan '02 program. Local energy conformations of the free acid were found using the Austin Model 1 (AM1) technique (Dewar, Zoebisch, Healey and Stewart, 1985) and resulting structures were used for the nickel (II) ion - laccaic acid A complex minimum energy structure using the Parameterized Model 3 (PM3) technique (Steware, 1989). Non-binding sites in the octahedral coordination shell of the nickel (II) ion were filled by water molecules. In putative structures where different numbers of water molecules were required, additional water molecules were added at non interacting distances to maintain constant mass.

5.3 Results and discussion

5.3.1 Effects of pH

Unsubstituted anthraquinone (9,10-anthraquinone) has a pale yellow colour and shows a weak absorption band at λ_{max} 405 nm (ε = 60) due to an n → π^* transition (Zollinger, 1991). Thai lac dye contains at least three laccaic acids (A, B and C), which are all anthraquinone derivatives. These acids contain electron-donating substituents (hydroxyl groups) in the α and β positions that cause a significant bathochromic shift observed at λ_{max} 487 nm. They are thus typical donor-acceptor systems in structure, with the carbonyl groups as the acceptors and the electron-releasing auxochromes as the donors (Zollinger, 1991; Christie, 2001).

The effect of pH on λ_{max} of the longest wavelength absorption band observed for Thai lac dye and Wako lac dye was very similar over the pH range 2.5-11 as shown in Figure 5.1. Over the pH range 2.5-6.0, there was only a small change in the λ_{max} value for the long wavelength maximum absorption in both dyes (λ_{max} 487 nm, pH 4.4 to λ_{max} 490 nm, pH 2.5). The oxygen atom of the quinone carbonyl groups in the 9- and 10- positions of the laccaic acid components of the dyes can not be protonated (Philipova, Ivanova, Kamdzhilov and Molina 2002) because of intramolecular hydrogen bond formation with the hydroxyl groups in the 1- and 4-positions respectively, thus explaining the insensitivity to pH change in the acidic region. At high pH values, the phenolic and carboxylic acid groups in the anthraquinone dye components would be deprotonated. Resultant charge delocalization in the phenolate anions would lead to stabilization of the excited state with lowering of the energy of the transition, thus giving rise to a pronounced

bathochromic shift (eg. λ_{max} 525 nm, pH = 9.9; Thai lac dye). This type of shift has been well documented with phenols (Philipova *et al.*, 2002; Crews and Rodriguez, 1998).

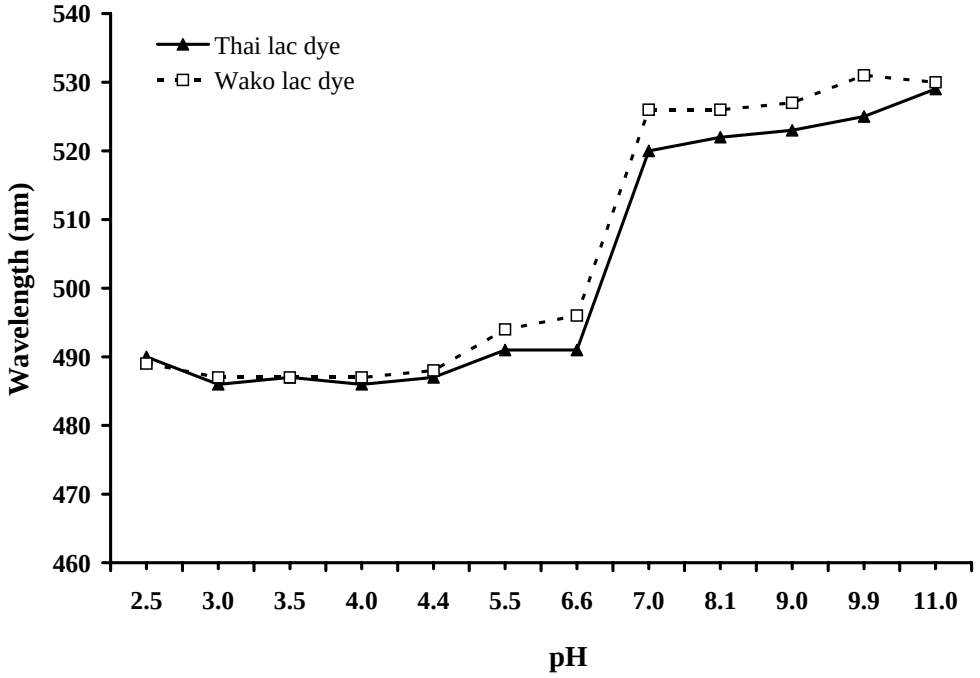


Figure 5.1 The effect of pH on the visible absorption band of Wako lac dye and Thai lac dye.

5.3.2 Metal ion effects

The UV-Visible spectra of Thai lac dye and Wako lac dye in the presence of metal ions are shown in Figure 5.2. Significant changes occurred in the bands absorbing at the longest wavelengths, and these changes were characteristic of the metal ion. With aluminum (III) ions, the λ_{max} value of the long wavelength absorption band was observed at 537 nm and with nickel (II) ions, two bands at λ_{max}

values of approximately 523 and 562 nm were observed. In the absence of metal ions, Thai lac dye itself had a long wavelength absorption maximum at 487 nm.

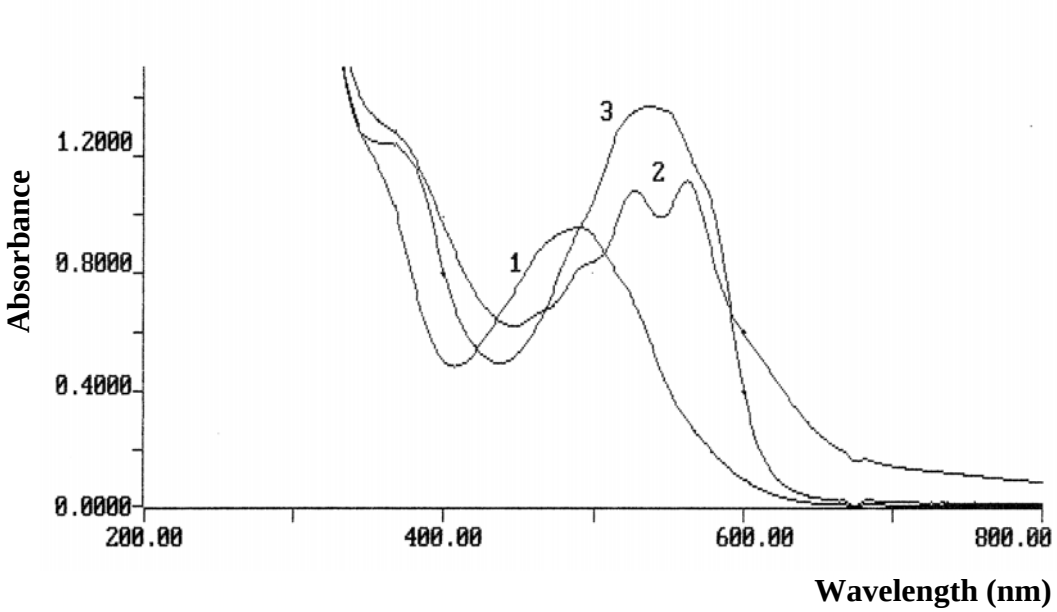


Figure 5.2 Absorption spectra of Thai lac dye solutions: (1) Thai lac dye (200 ppm); (2) Mixture of Thai lac dye (200 ppm) and nickel (II) ion ($1x10^{-2}$ M); (3) Mixture of Thai lac dye (200 ppm) and alum ($1x10^{-2}$ M).

Metal ions also caused a bathochromic shift of the long wavelength absorption bands of laccaic acids A and B themselves with increasing ion concentrations, for example with alum (Figure 5.3). It was also observed that there was little change in pH (ca 4.0) over alum concentration ranges of $5.0x10^{-5}$ M to $4.0x10^{-2}$ M (Table 5.1). It is probable that the bathochromic shift observed with laccaic acids A and B, as with other dyes (Christie, 2001), occurs as a result of coordination by the lone pair electrons on the N or O donor atoms with the aluminum ion site, thus stabilizing the excited state relative to the ground state leading to longer wavelength absorption maxima (Philipova *et al*., 2002; Crews and Rodriguez, 1998).

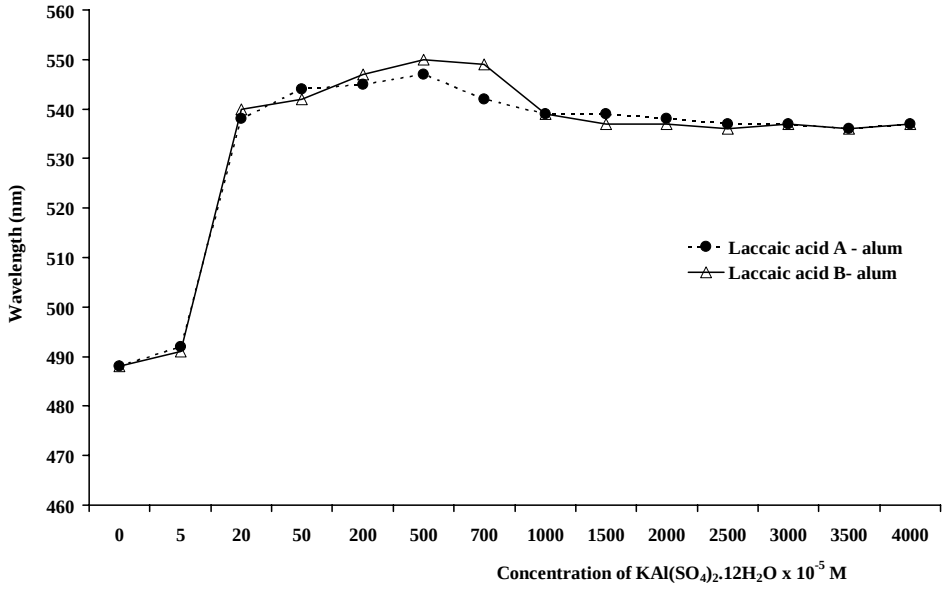


Figure 5.3 The effect of alum concentrations on the most intense visible absorption band of laccaic acids A and B.

Table 5.1 Changes in solutions of laccaic acids with alum and nickel (II) ion

Solution	Laccaic acid concentration (M)	Ion concentration (M)	pH
Laccaic acid A – alum	9.31×10^{-5}	$5.0 \times 10^{-5} – 4.0 \times 10^{-2}$	4.45-3.90
Laccaic acid B – alum	1.05×10^{-4}	$5.0 \times 10^{-5} – 4.0 \times 10^{-2}$	4.58-3.97
Laccaic acid A – Ni(II)	9.31×10^{-5}	$5.0 \times 10^{-5} – 4.0 \times 10^{-2}$	4.90-4.44
Laccaic acid B – Ni(II)	1.05×10^{-4}	$5.0 \times 10^{-5} – 4.0 \times 10^{-2}$	5.00-4.37

In addition, an increase of the absorption intensity within the range of alum concentration was observed as shown in Table 5.2. This behaviour of laccaic acids is similar to the other dyes (Christie, 2001) where the increased absorption intensity plateaued at higher concentrations.

Table 5.2 Absorbance value dependence of laccaic acids A and B on concentrations of alum at λ = 537 nm and nickel (II) ion at λ = 562 nm

Alum concentrations x10^{-5} (M)	Absorbance of laccaic acid A – alum	Absorbance of laccaic acid B – alum	Ni(II) ion concentrations x10^{-5} (M)	Absorbance of laccaic acid A – Ni(II)	Absorbance of laccaic acid B – Ni(II)
0	0.4621	0.4386	0	0.1257	0.1123
5	0.6526	0.6011	5	0.2022	0.1313
20	0.9887	1.0098	20	0.2598	0.2821
50	1.0025	1.1130	50	0.3866	0.2991
200	1.1789	1.2335	200	0.5222	0.5230
500	1.3032	1.3727	500	0.6424	0.7365
700	1.2960	1.4284	700	0.7620	0.7689
1000	1.3832	1.4615	1000	0.8140	0.8004
1500	1.4121	1.4547	1500	0.8655	0.8784
2000	1.4371	1.4916	2000	0.9090	1.0428
2500	1.4639	1.5357	2500	1.0254	0.9936
3000	1.4880	1.5156	3000	0.9979	1.0056
3500	1.5205	1.5034	3500	1.0526	1.0272
4000	1.4927	1.5391	4000	1.0771	1.0807

The effect of varying alum concentrations on Thai lac dye compared to Wako lac dye absorption spectra is presented in Figure 5.4, and a similar bathochromic shift of the lac dye band was observed as for the laccaic acids. This result is consistent with these acids being the major components of the dyes. A greater absorbance difference between the dyes was observed on monitoring at λ 537 nm (Table 5.3). It is apparent that Thai lac dye, which was not as pure as Wako lac dye, absorbed less strongly. Furthermore, the pH of the Thai lac dye solution was consistently higher than Wako lac dye at all alum concentrations (Table 5.4).

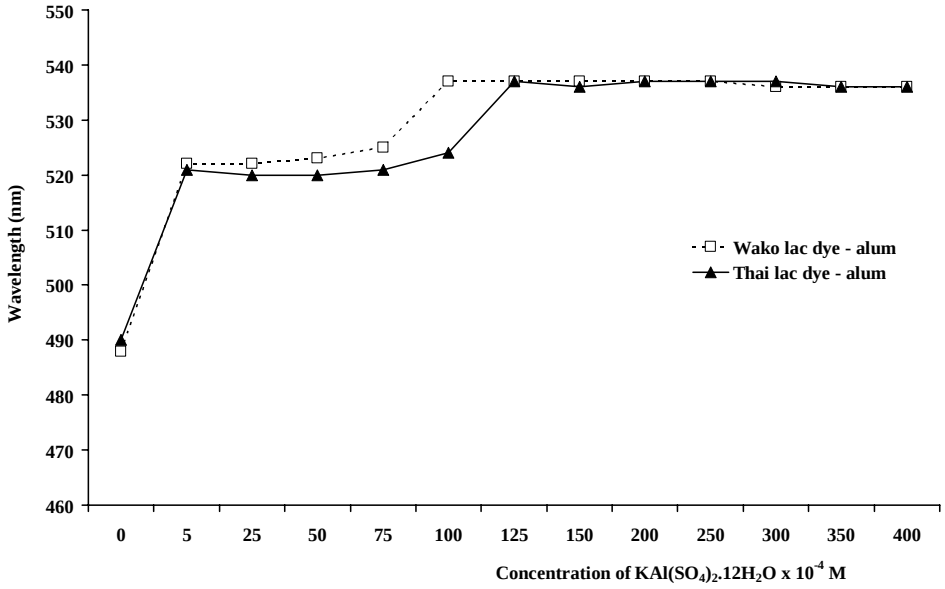


Figure 5.4 The effect of alum concentrations on the most intense visible absorption band of Wako and Thai lac dye.

Table 5.3 Absorbance value dependence of Thai lac dye and Wako lac dye on concentrations of alum at λ = 537 nm and nickel (II) ion at λ = 562 nm

Alum concentrations x10^{-4} (M)	Absorbance of Thai lac dye-alum	Absorbance of Wako lac dye – alum	Ni(II) ion concentrations x10^{-4} (M)	Absorbance of Thai lac dye –Ni(II)	Absorbance of Wako lac dye – Ni(II)
0	0.1085	0.3068	0	0.0503	0.0633
5	0.2470	0.7192	5	0.2253	0.1968
25	0.2737	0.8502	25	0.2467	0.3626
50	0.2906	0.8966	50	0.2407	0.4451
75	0.2890	0.9064	75	0.2444	0.4933
100	0.2918	0.9412	100	0.2502	0.5041
125	0.2908	0.9479	125	0.2540	0.5404
150	0.3037	0.9548	150	0.2651	0.6035
200	0.2989	0.9421	200	0.2715	0.6870
250	0.3004	0.9917	250	0.2672	0.6837
300	0.3022	0.9726	300	0.2655	0.7694
350	0.3090	1.0245	350	0.2723	0.7521
400	0.3139	1.0382	400	0.2724	0.7373

Table 5.4 Changes in pH of solutions of Wako lac dye and Thai lac dye with alum and nickel (II) ion

Solution	Lac dye concentration (ppm)	Ion concentration (M)	pH
Wako lac dye – alum	49	$5.0x10^{-4} – 4.0x10^{-2}$	3.73-3.32
Thai lac dye – alum	50	$5.0x10^{-5} – 4.0x10^{-2}$	4.10-3.37
Wako lac dye – Ni(II)	49	$5.0x10^{-5} – 4.0x10^{-2}$	4.39-4.02
Thai lac dye – Ni(II)	50	$5.0x10^{-5} – 4.0x10^{-2}$	5.63-5.12

The results of the study of nickel (II) ion effects on the visible spectra (λ_{max}) of laccaic acids A and B and the lac dyes are presented in Figures 5.5 and 5.6 which indicated the larger bathochromic shift of laccaic acids A and B as the metal ion concentration increased. This behaviour was also observed in Wako lac dye and Thai lac dye. The higher metal ion concentration presumably results in stronger interaction with the dyes stabilizing the excited state and leading to a lower energy separation from the ground state. When the concentration of nickel (II) ions increased within the range of concentrations studied, the intensity of the long wavelength absorption band of laccaic acids A and B and Wako lac dye increased except in the case of Thai lac dye which was not much affected as shown in Tables 5.2 and 5.3. The different behaviour of Thai lac dye from laccaic acids A and B and Wako lac dye, was consistent with the lower purity of Thai lac dye hence the lower absorption. The similar pattern of behaviour of laccaic acids A and B and the lac dyes again indicated similar chromophores for these substances. These visible absorbance results differ in some respects from the interactions of other dyes with metal ions (Christie, 2001) where the increased absorption intensity plateaued at higher concentrations.

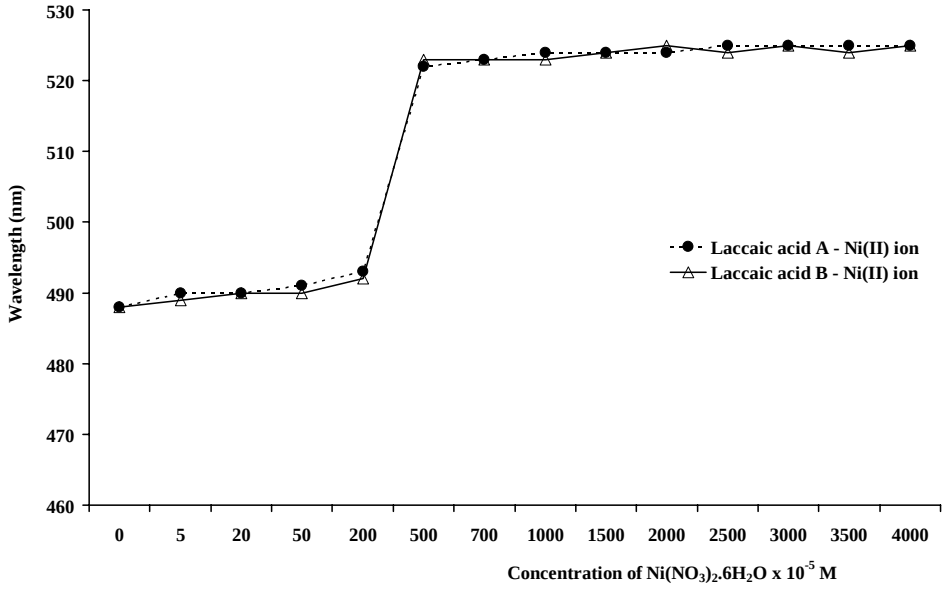


Figure 5.5 The effect of nickel (II) ion concentrations on the visible absorption band of laccaic acids A and B.

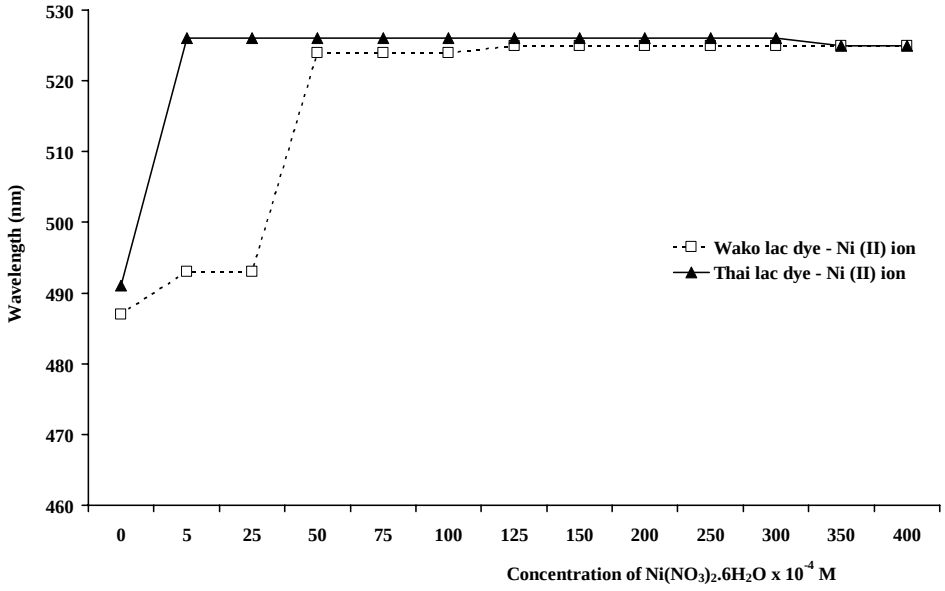


Figure 5.6 The effect of nickel (II) ion concentrations on the visible absorption band of Wako lac dye and Thai lac dye.

5.3.3 Nickel (II) – laccaic acid A complex

The mole ratio of nickel (II): laccaic acid A as determined by Job's method (Figure 5.7) was 1:1 and it is likely that the rest of the coordination sites of nickel (II) ion would be occupied by solvent molecules. Computer-based molecular modelling studies using the Spartan '02 computer program (AM1/PM3; ′02 Linux/Unix) were then undertaken to assess possible structures for this 1:1 nickel (II) – laccaic acid A complex. In the modelling process, water was included as a ligand, and the phenolic and carboxylic acid groups were not ionized since in the experimental study the solution was in the pH range 2.8-4.2. The complex shown in Figure 5.8 was the one with the lowest heat of formation (-195 kJ/mol) and it incorporated the quinone carbonyl oxygen and the phenolic group as ligands and four water ligands in the octahedral complex. Interestingly, the complex structure was also stabilized by hydrogen bonding interactions with the carboxylic acid functionality, and a phenolic group and the acetamide carbonyl group in the aryl substituent. These interactions, together with increased electron density at the quinone carbonyl oxygen from lone pair electron delocalization from the phenolic groups at positions 3 and 6, presumably account for this preference for nickel ion complex formation at the position proposed. In support of the latter point, an electrostatic potential map (Spartan program, AM1) of laccaic acid A confirmed higher negative potential at the 9-quinone carbonyl oxygen group compared with the potential at the other quinone group.

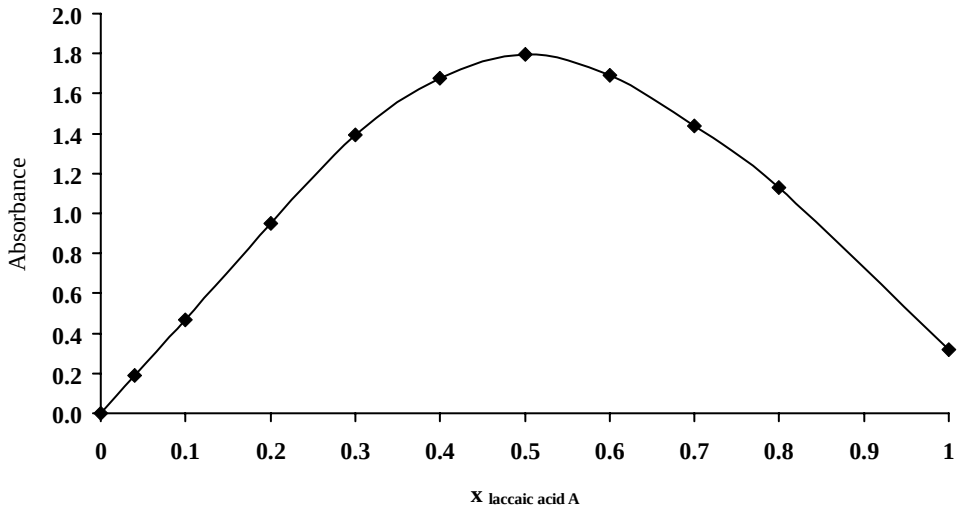


Figure 5.7 Determination of the mole ratio for nickel (II) ion-laccaic acid A complex in ethanol.

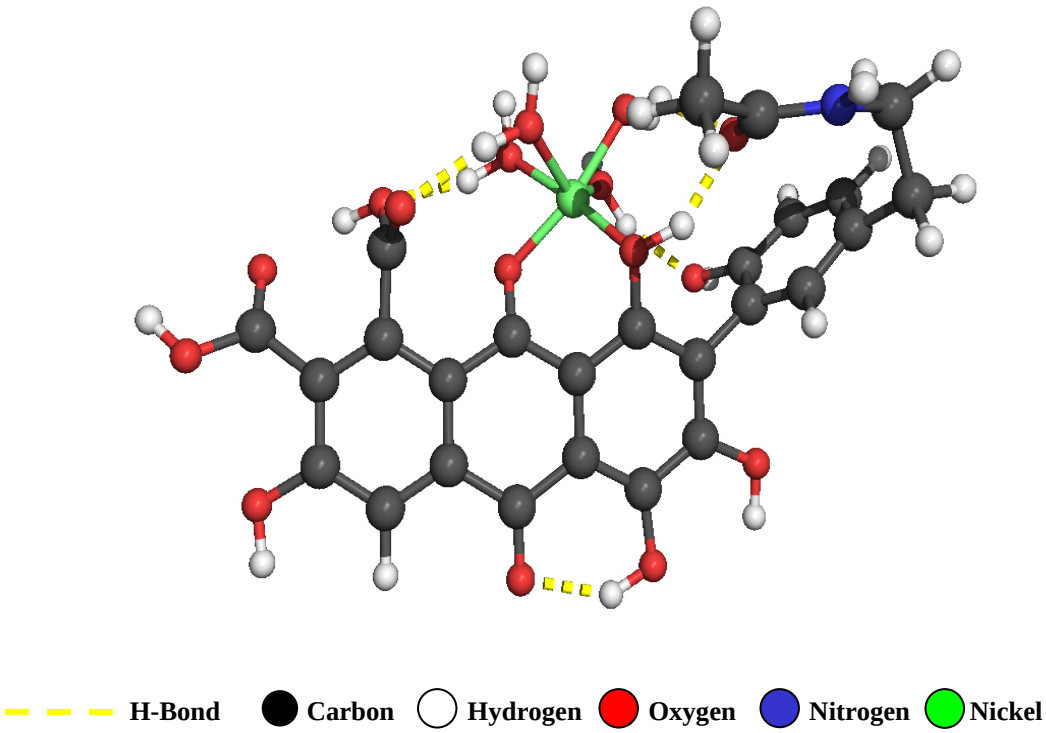


Figure 5.8 The proposed structure of nickel (II) ion – laccaic acid A complex from computer-based modelling (Spartan '02 Linux/Unix; PM3).

5.4 Conclusions

When the pH of the lac dye solution was increased from 2.5 to 11, a substantial bathochromic shift of the lac dye visible absorption band was observed in both Wako and Thai lac dyes. Also, increasing the metal ion concentration, for both alum and nickel (II) ion, caused a bathochromic shift of the visible absorption bands in both dye samples (and in their laccaic acid components, laccaic acids A and B). The bathochromic shifts observed are consistent with the lone pair electrons in the donor atoms (N and O in the lac dyes/laccaic acids) participating in metal ion coordination and stabilizing the excited state relative to the ground state. Support for this participation was obtained from a modelling study on the proposed nickel (II) – laccaic acid A complex. However, the metal ions have not been used as mordants in this research because some metal ions may effect the environment and public health. Chitosan, a substance derived from the natural polymer found in the shell of crabs and shrimps (Majeti and Ravi, 2000), was used to treat silk and cotton in order to enhance the adsorption of lac dyeing in this research.

CHAPTER VI

CONCLUSIONS

The extraction and characterization of lac dye from Thai stick lac and the development of lac dyeing on silk and cotton were carried out in this research project.

Ultrasonication and microwave-assisted methods were used to extract lac dye from Thai stick lac. These results were compared to those from the hot water (60-75 ^{o}C) extraction method. It was found that the total percentage of laccaic acid obtained by the ultrasonication and microwave-assisted extraction methods was higher than that from the hot water extraction method. It was also shown that the ultrasonication and microwave extraction methods increased the solubility of lac dye in water. The total percentage of laccaic acid represented about 4.84, 5.70 and 6.12% by weight of lac dye extracted by using the hot water, ultrasonication and microwave extraction methods respectively. As also reported by other workers (Burwood *et al*., 1965; Oka *et al*., 1998), the major component obtained from the three extraction methods by column chromatography was laccaic acid A with the minor laccaic acids being B and C, especially from the microwave extraction. In addition, the percentage of laccaic acid B obtained by the hot water and ultrasonication extraction methods were essentially the same. These results were not substantial, however, a slight improvement in the percentage of laccaic acids obtained was observed using the new extraction methods (the ultrasonication and microwave-assisted extraction methods) previously unreported in the literature, compared to the traditional extraction method

(hot water) however no new minor components in the lac dye were detected on using these new extraction methods. The total percentage of laccaic acid obtained from Thai stick lac from Nakhon Ratchasima and Surin provinces was 6.47% and 4.84% respectively, which was consistent with regional variations in the quantity and composition of lac components.

The adsorption kinetics and thermodynamics of lac dyeing onto silk and cotton fibres were investigated in this study. The adsorption capacities are significantly affected by pH, the initial dye concentration, the material to liquor ratio (MLR) and temperature. It was found that the dye uptake increased with decreasing pH. Also, the dye uptake increased at higher initial dye concentration and with a higher material to liquor ratio (MLR). In addition, the initial dye adsorption rates (h_i) onto the cotton before equilibrium was reached increased at higher dyeing temperatures. The pseudo second-order kinetic model was indicated for both lac dyeing onto silk and cotton fibres at pH 3.0 with activation energies (E_a) of 47.0 and 42.4 kJ/mol respectively. Also, batch isotherm studies showed that the adsorption of lac dye on silk and cotton fibres at pH 3.0 was described by the Langmuir and Freundlich isotherms respectively. However, the enthalpy (ΔH^o) for lac dyeing on silk was higher than that dyeing on cotton. The values of the enthalpy for the lac dyeing of silk and cotton fibres at pH 3.0 were -31.4 and -4.88 kJ/mol respectively. The small negative value of enthalpy (ΔH^o) suggested that the adsorption of lac dye on cotton was a physical process. The results indicated that lac dye has a greater affinity for silk than cotton. The low affinity of lac dye for cotton fabric is due to fewer binding sites on cotton for the dye attachment. To enhance the dye uptake in both fibres, a 0.3% (v/v) aqueous solution of chitosan was used to treat the silk and cotton fibres. It was

found that the pretreatment of the fibres with chitosan increased the dye adsorbed on the fibres and also decreased the amount of dye desorbed from the fibres compared to the untreated ones. The effect of NaCl on adsorption and desorption of lac dyeing onto cotton was also studied. It was found that NaCl increased the adsorption ability of lac dye on cotton without pH control. However, the lac dye could be more easily desorbed from the cotton.

As part of an investigation on mordants and lac dyeing of silk and cotton, UV-VIS spectroscopic studies were carried out on the effect of pH and metal ions on Thai lac dye extracted from stick lac from the Rain tree in northeast Thailand. These results were compared with those from commercial lac dye (Wako Company), and from laccaic acids A and B. It was shown that increasing the metal ion concentration caused a bathochromic shift in the lac dye absorption bands in the visible region in both the laccaic acids A and B and the dyes. Also when the pH of the lac dye solution was increased from 2.5 to 11, a substantial bathochromic shift of the lac dye visible absorption band was observed in both Wako and Thai lac dyes. At alkaline pH, the phenolic hydroxyl (and carboxylic acid groups) in the lac dye components are deprotonated, resulting in a pronounced bathochromic shift. The study also indicated that when the concentration of metal salts increased within the range of the concentrations studied, the intensity of the long wavelength absorption band of laccaic acid A, laccaic acid B and commercial lac dye increased accordingly. However, the metal ions were not then used as mordants in this study because of the potential negative environment and public health effects due to their toxicity.

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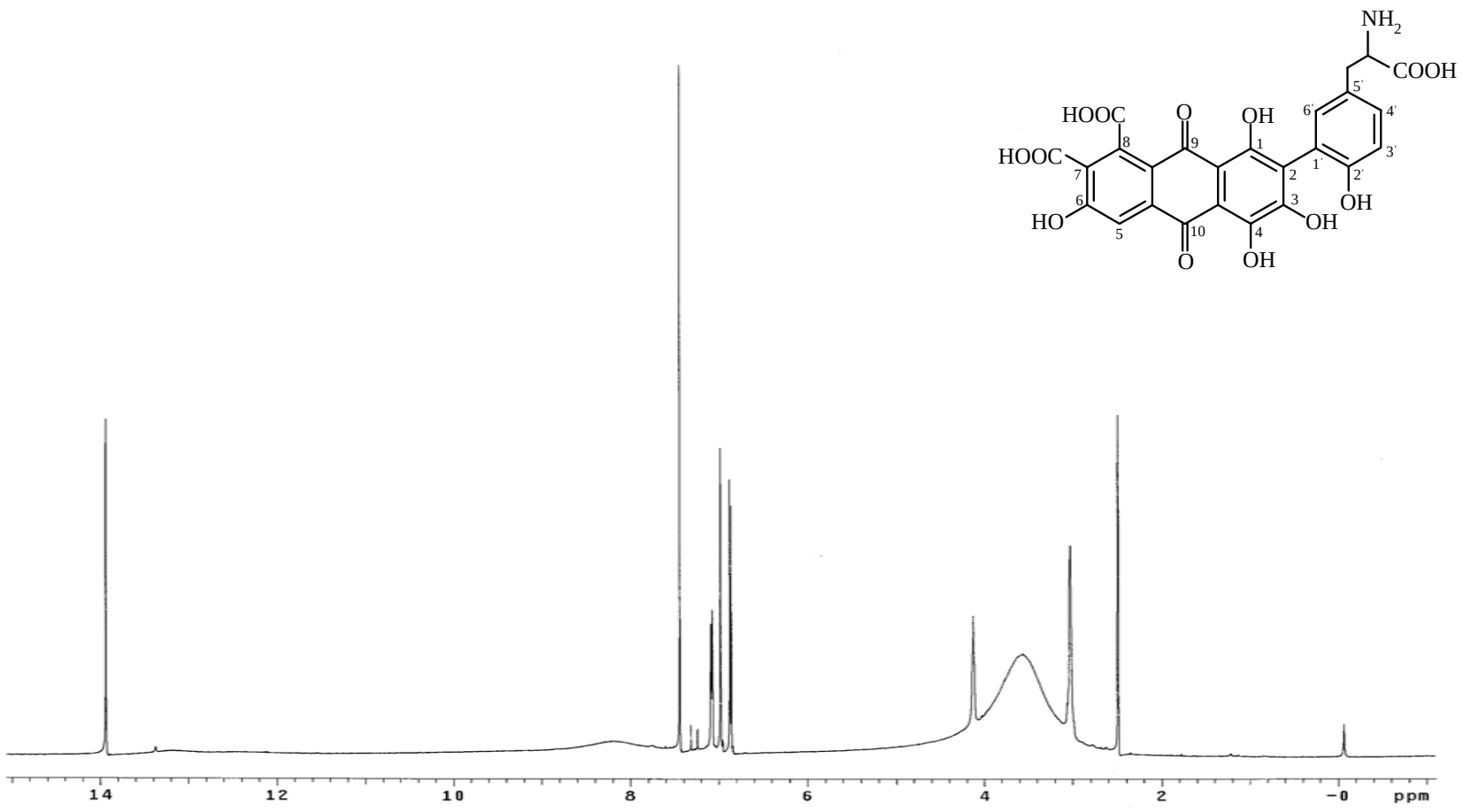
APPENDICES

APPENDIX A

^{1}H- and ^{13}C-NMR Spectra



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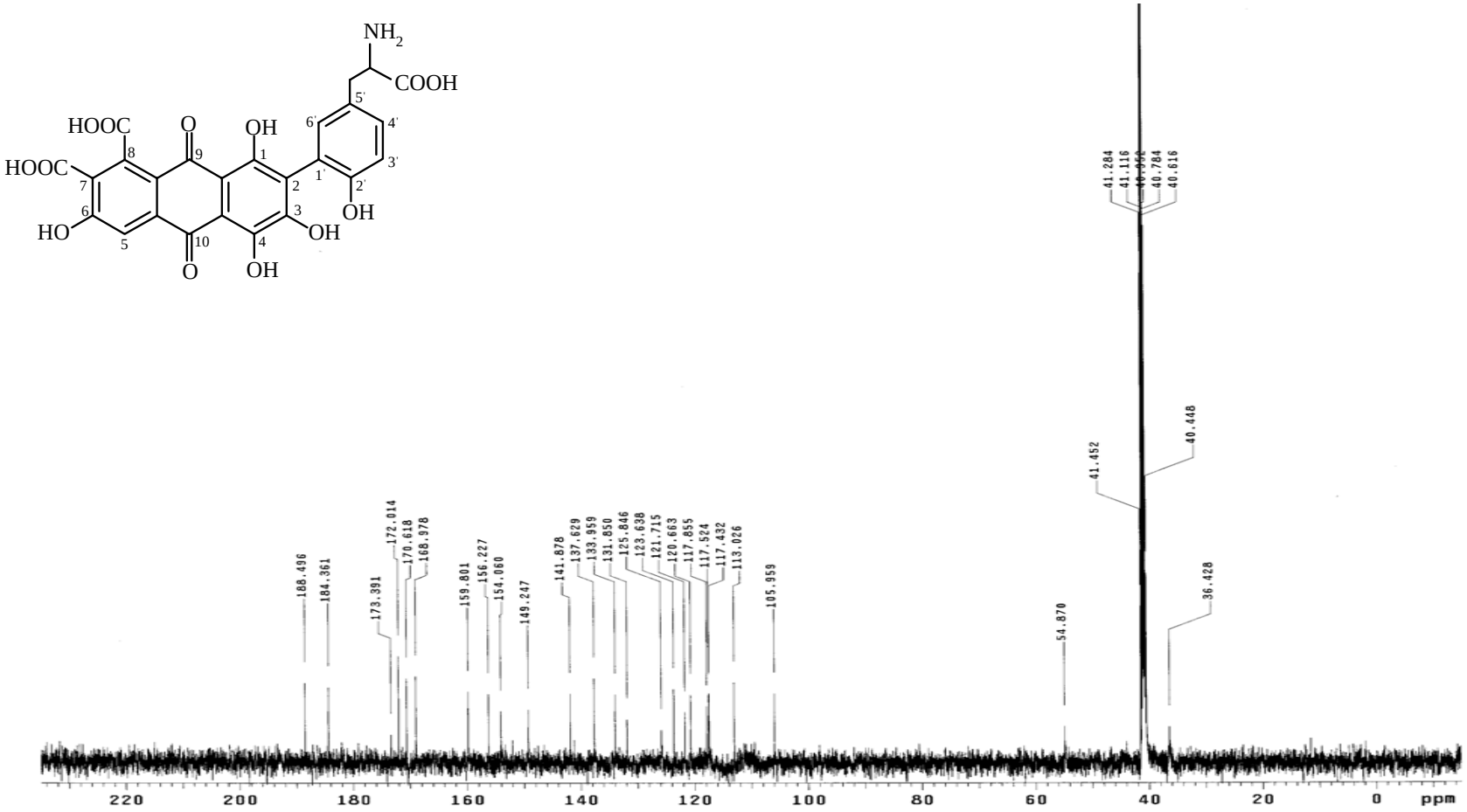

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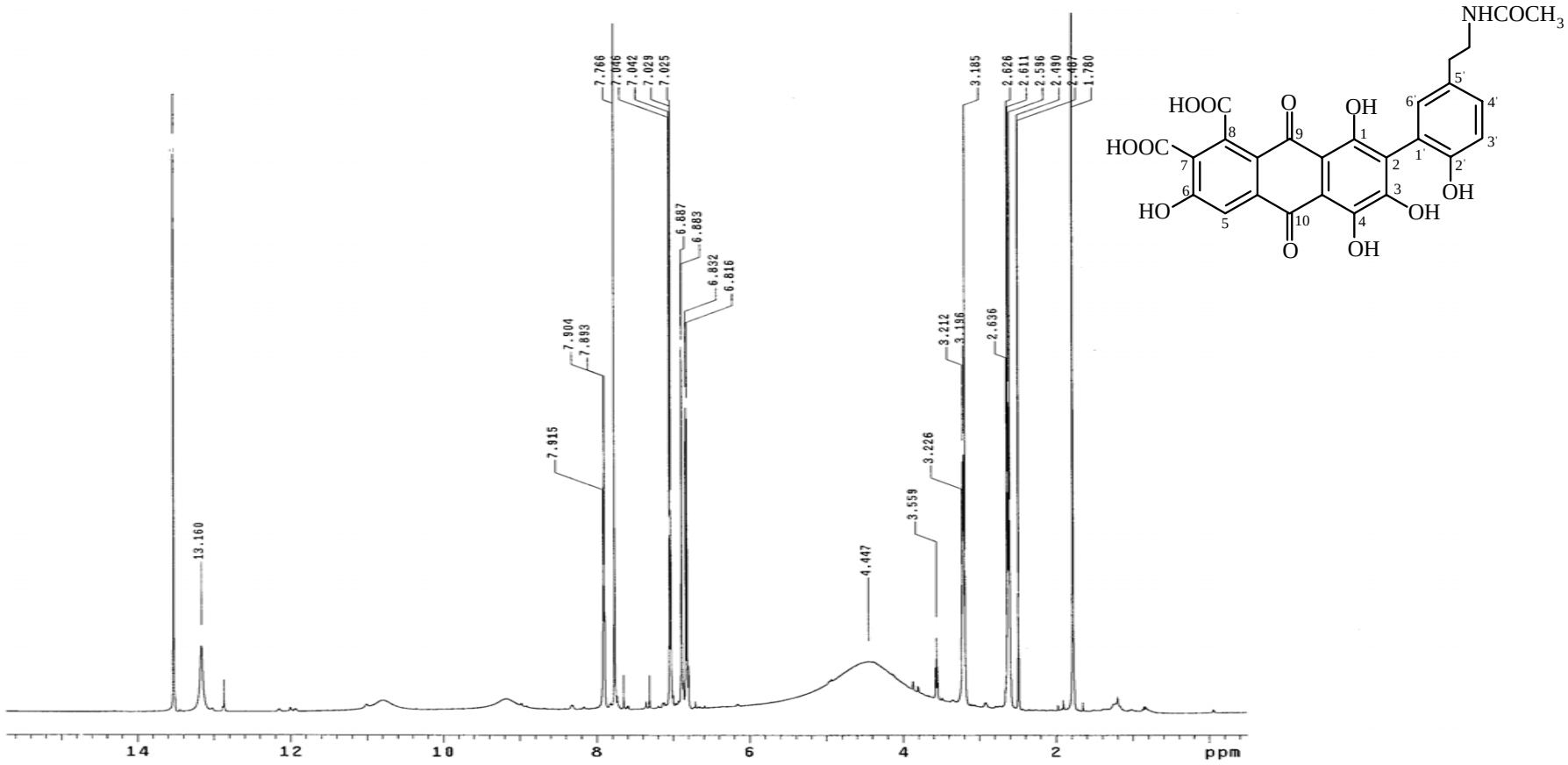

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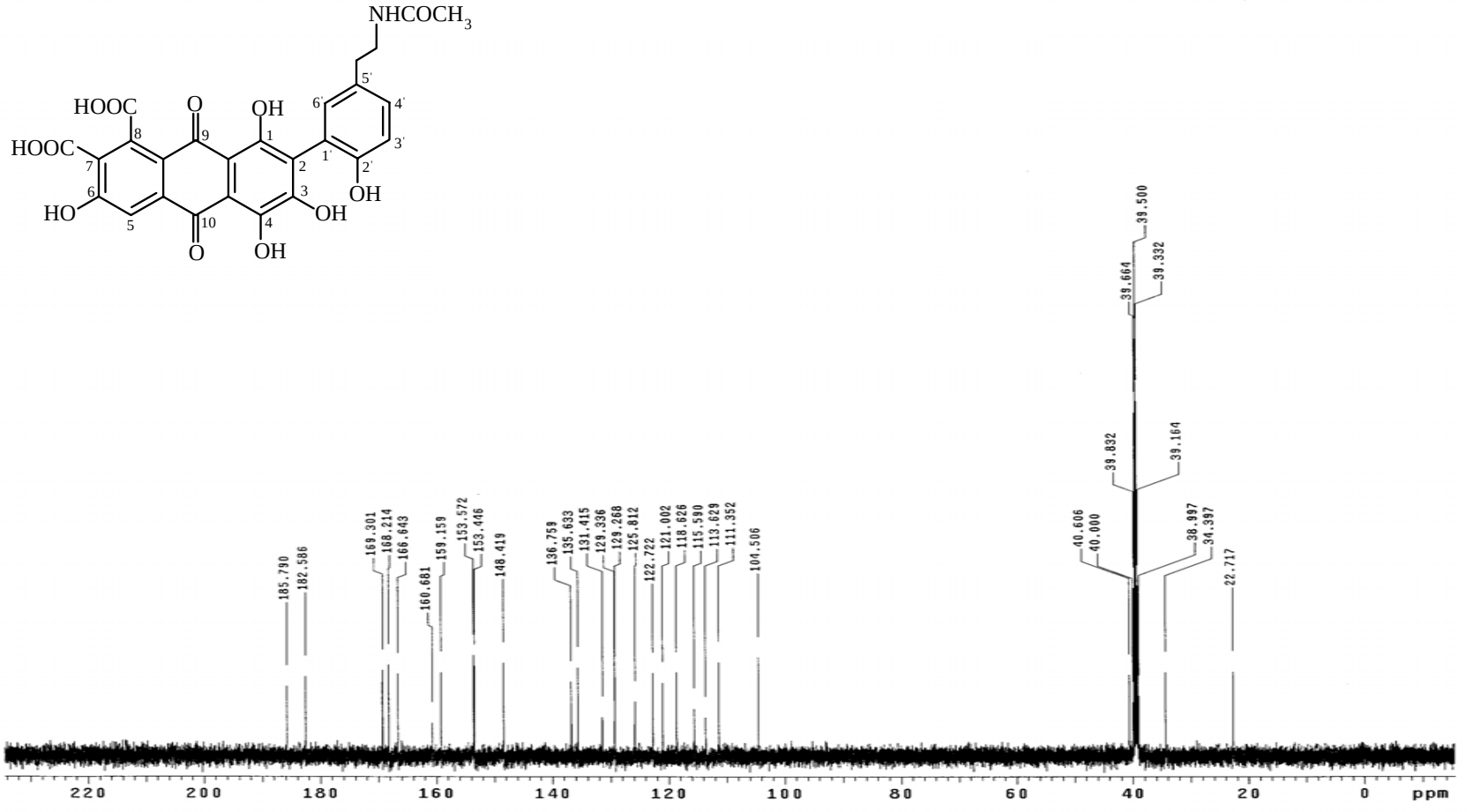

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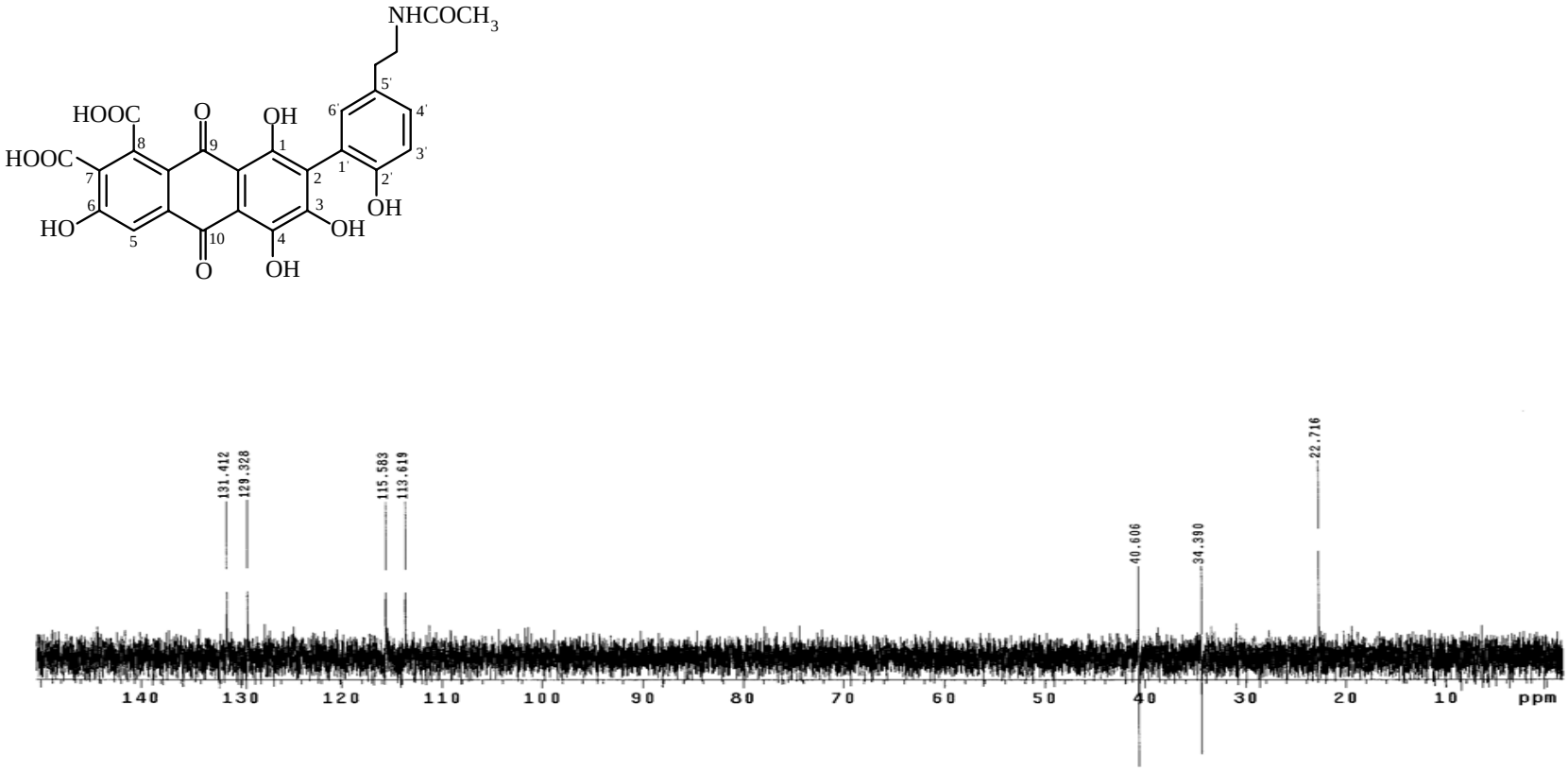

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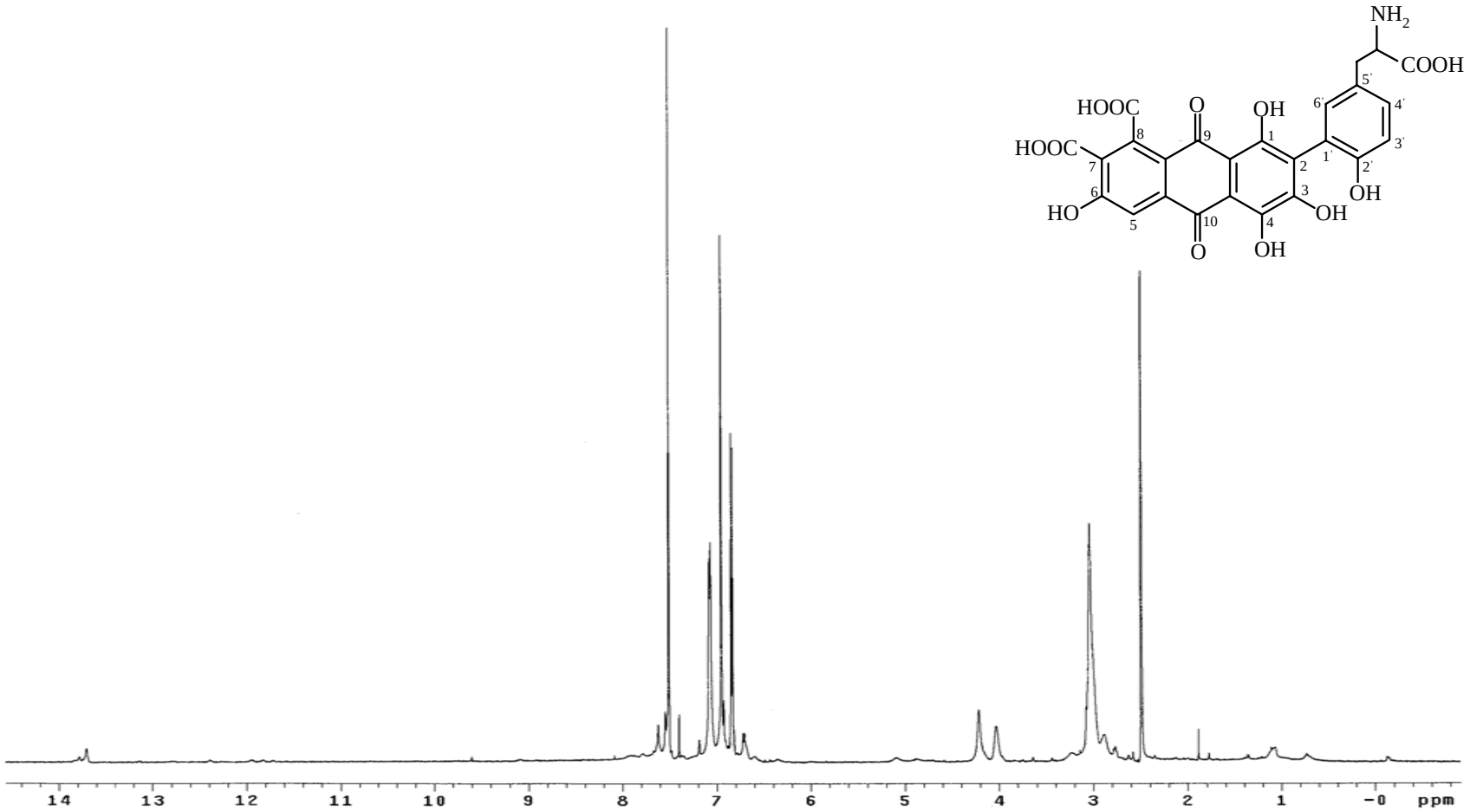

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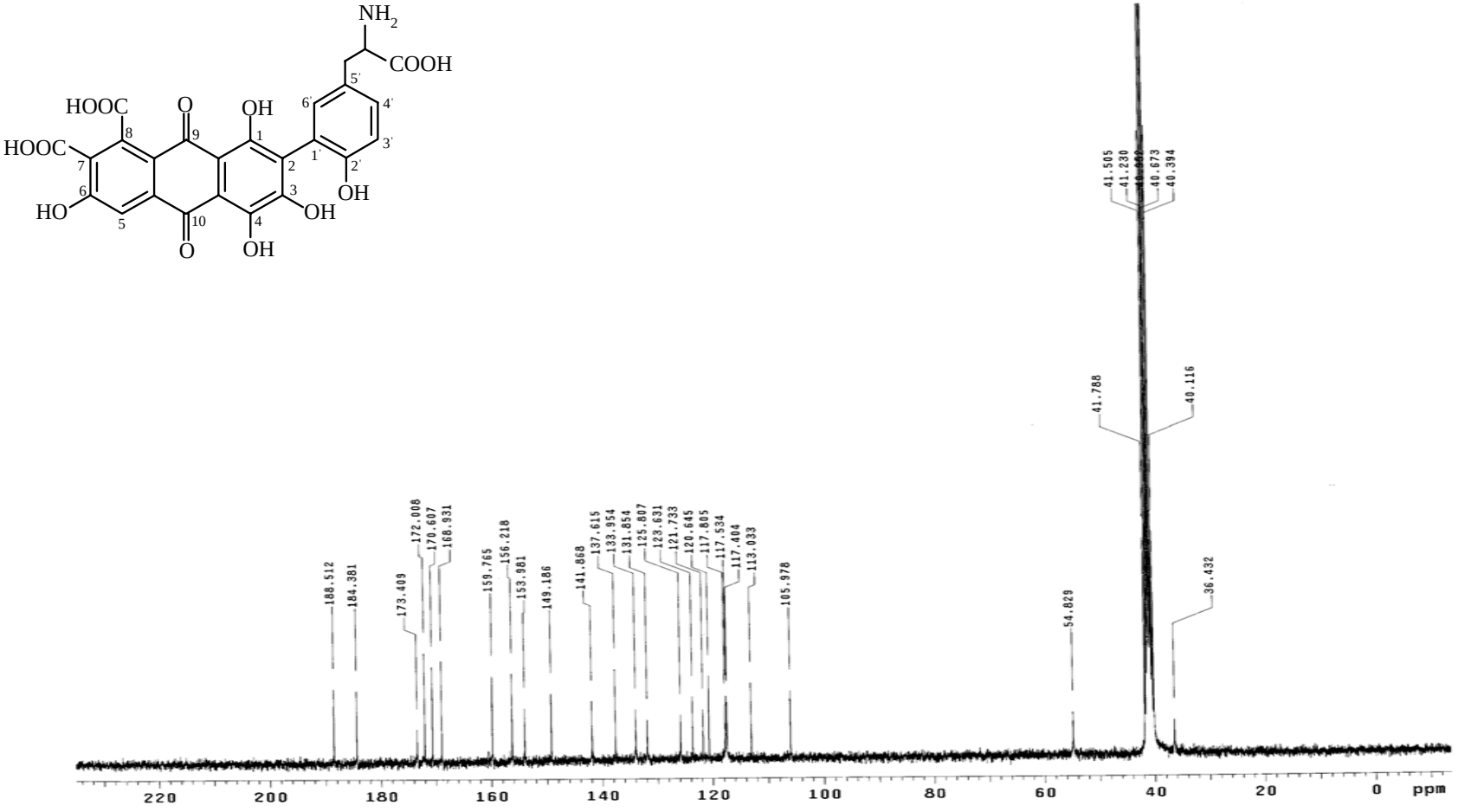

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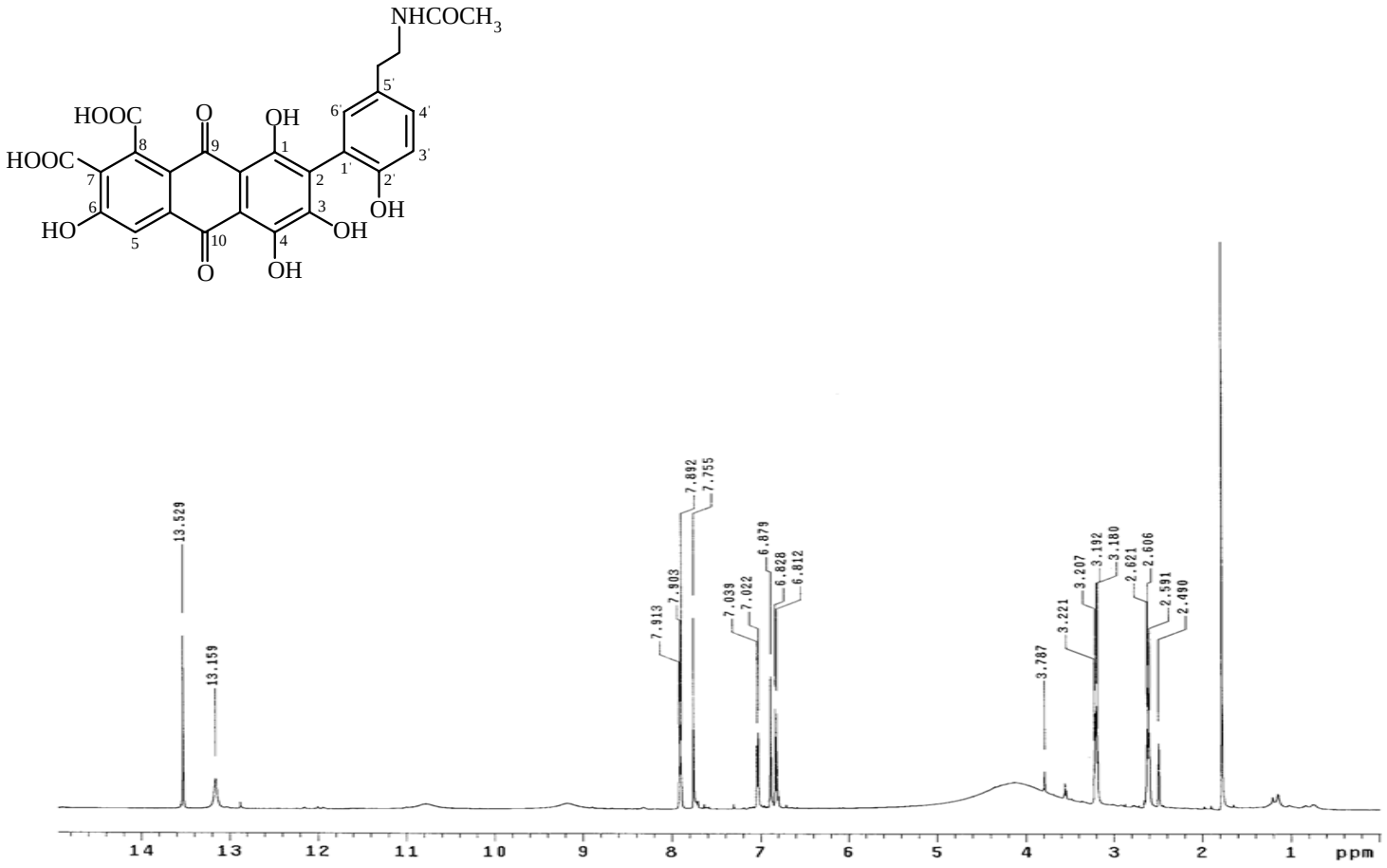
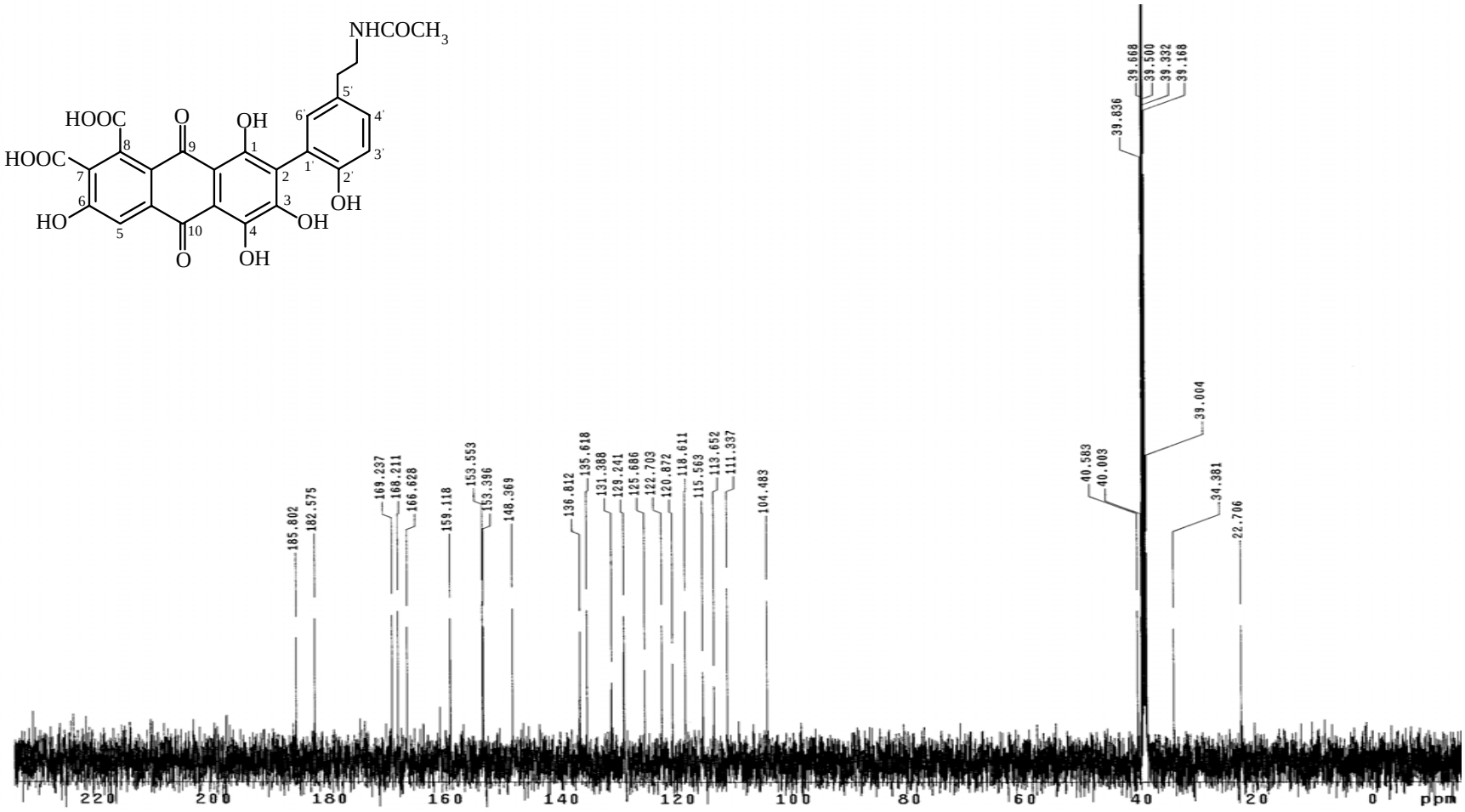


[1]H-NMR spectrum of fraction 1 from the hot water extract from Nakhon Ratchasima province in DMSO – d6



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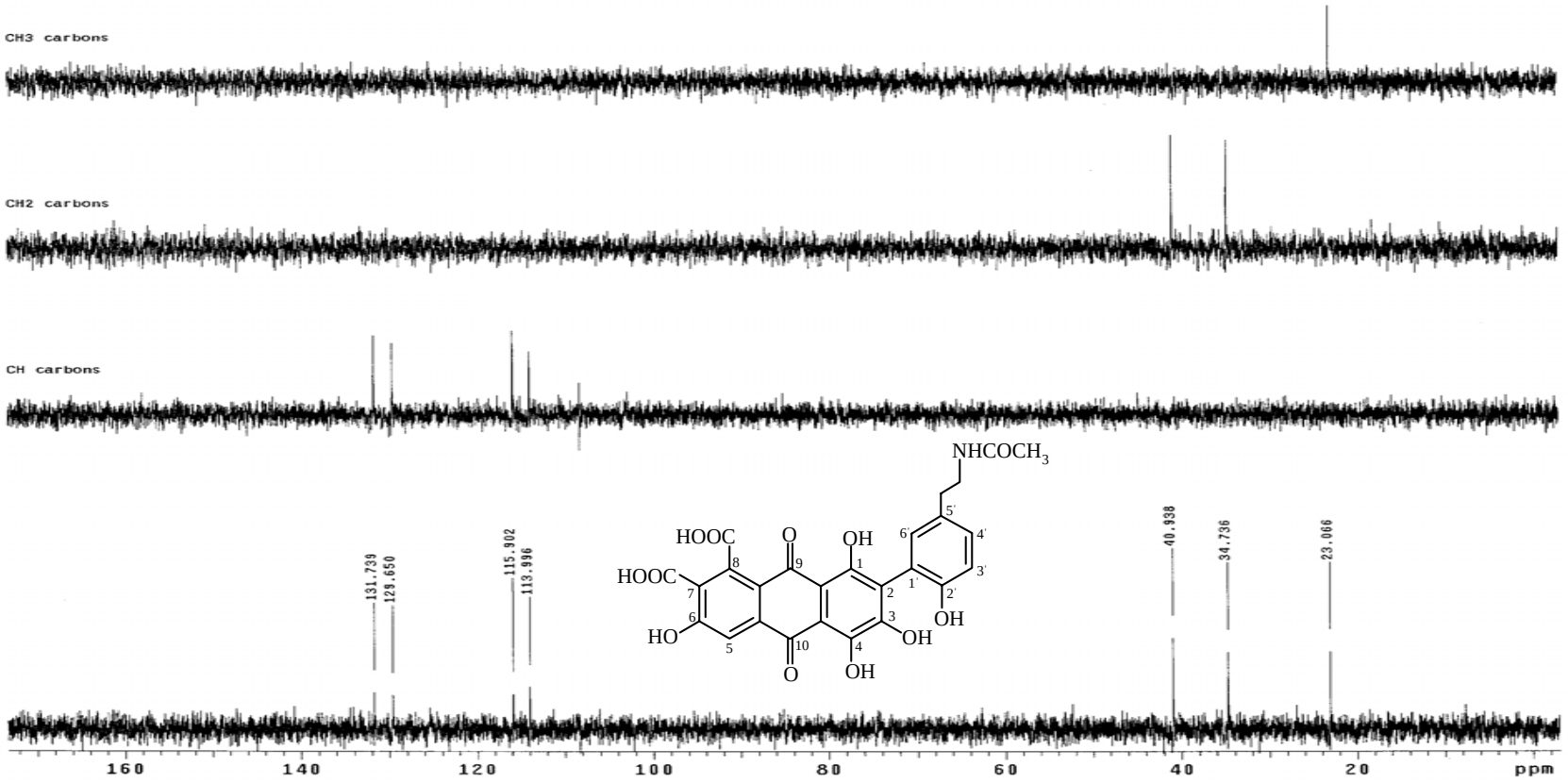

DEPT spectrum of fraction 1 from the hot water extract from Nakhon Ratchasima province in DMSO – d6

APPENDIX B

Publication

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An absorption spectroscopic investigation of the interaction of lac dyes with metal ions

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Abstract

As part of an investigation on mordants and lac dyeing of silk and cotton, UV–VIS spectroscopic studies we carried out on the effect of pH and metal ions on Thai lac dye extracted from stick lac from the Rain tree in northea Thailand. These results were compared with those from commercial lac dye (Wako Company), and from laccaic acids and B. It was shown that increasing the metal ion concentration caused a bathochromic shift of the lac dye absorptic bands in the visible region in both the laccaic acids A and B and the dyes. Also when the pH of the lac dye solution w increased from 2.5 to 11, a substantial bathochromic shift of the lac dye visible absorption band was observed in bo Wako and Thai lac dyes. At alkaline pH, the phenolic hydroxyl (and carboxylic acid groups) in lac dye molecules deprotonated, resulting in a pronounced bathochromic shift. The study also indicated that when the concentration metal salts increased within the range of concentrations studied, the intensity of the long wavelength absorption band laccaic acid A, laccaic acid B and commercial lac dye increased accordingly.

Keywords: Lac dye; Thai lac dye; Absorption spectroscopy; Metal ions; Rain tree; Laccaic acid

1. Introduction

Lac, a natural resin of insect origin, is used extensively for natural food additives, cosmetics and as a colourant for silk and cotton dyeing. Lac dye, which is the soluble part of stick lac, is composed mainly of two major anthraquinone-based con ponents: laccaic acids A and B [1 7]; the min components, laccaic acids C, D and E, have als been isolated [8,9] (Fig. 1). The UV VIS spectru of laccaic acid A is very similar to the spectrum crude laccaic acid in ethanol [2], with absorptic maxima for laccaic acid A reported at 290, 34 500 and 530 nm in this solvent [2]; in water, λ_m values were reported at 292 and 490 nm and 500 nm in acetone for this acid (referred to laccaic acid (I)) [10].

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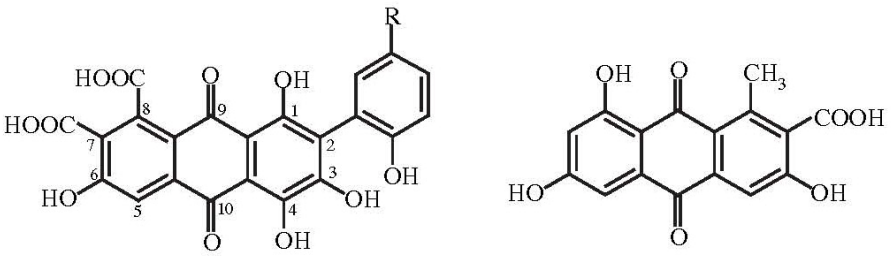

Laccaic acid A R = $CH_2CH_2NHCOCH_3$ MW 537

Laccaic acid B R = CH_2CH_2OH MW 496

Laccaic acid C R = $CH_2CHCOOH$ (with NH_2) MW 539

Laccaic acid E R = $CH_2CH_2NH_2$ MW 495

Laccaic acid D MW 314

Fig. 1. Chemical structures of laccaic acids.

For generations, villagers in northeast Thailand have used lac dyes extracted from stick lac formed on the Rain tree, *Samanea saman* (Jacq.) Merr. (*Pithecolobium saman*, Mimosaceae) for textile dyeing [11]. As part of the dyeing process with lac dye on cotton and silk, alum, potassium dichromate, stannous chloride and copper sulfate have been used as mordants to improve colour fastness. While metal ion mordants have also been used by others in dyeing with lac dyes [12 14], there have not been any reports on specific spectroscopic studies involving these components. In an effort to gain a greater understanding of the action of these mordants, and with a view ultimately to improving the reliability and quality of the dyeing process, a systematic study of the interaction of lac dyes and laccaic acids with selected metal ions was undertaken. In particular, metal ion and pH effects on the visible absorption spectra of Thai lac dye extracted from stick lac from the Rain tree in northeast Thailand were investigated and comparative studies done with commercial lac dye (Wako Company) and laccaic acids A and B in order to ascertain if the Thai lac dye had any distinctively different characteristics. The results of this study are reported in this paper.

2. Experimental

2.1. Chemicals

Laccaic acid A [124-04791], laccaic acid B [127-04801], and lac dye [124-04012] were purchased from the Wako Company (Osaka, Japan). For simplicity, the last compound is referred to as Wako lac dye. Analytical grade metal ion salts, $Ni(NO_3)_2{\cdot}6H_2O$ and $KAl(SO_4)_2{\cdot}12H_2O$ (alum) were purchased from the Merck Co., Ltd.

2.2. Extraction of lac dye

Stick lac (500.48 g) collected from Rain trees in northeast Thailand (Nakhon Ratchasima Province) was powdered in a grinding mill and finely ground (18 mesh). The powdered material was extracted with deionized water (1.5 L) at 60 °C for 1 h. The aqueous solution was filtered and then concentrated under reduced pressure (rotary evaporator) to give a crude lac dye extract (38.33 g), which was then used without further purification. This extract is referred to as Thai lac dye.

2.3. Methods

2.3.1. Measurement of pH and spectra

The influence of pH on the visible absorption spectra of Wako lac dye and Thai lac dye was determined using 3% (v/v) acetic acid solution in the pH range 2.5 4.5, and 0.5 M sodium hydroxide solution in the pH range 5.0 11.0. The pH values were measured using a pH meter (Mettler Delta 320, UK). The UV VIS spectra were recorded using a Cary 1E UV VIS spectrophotometer. This instrument was used for all UV VIS absorption spectra in this work.

2.3.2. Metal ion effects

2.3.2.1. Effect of metal ion concentration on laccaic acid A and laccaic acid B visible absorption spectra in deionized water. The final concentration of laccaic acids A and B in the measured aqueous solutions (deionized water) was 9.31×10^{-5} and 1.05×10^{-4} M, respectively. The final concentrations of metal ions in the laccaic acids A and B solution varied as follows: 5.0×10^{-5}, 2.0×10^{-4}, 5.0×10^{-4}, 2.0×10^{-3}, 5.0×10^{-3}, 7.0×10^{-3}, 1.0×10^{-2}, 1.5×10^{-2}, 2.0×10^{-2}, 2.5×10^{-2}, 3.0×10^{-2}, 3.5×10^{-2}, and 4.0×10^{-2} M. The pH of the final solution in each case was measured using a pH meter.

2.3.2.2. Alum solution studies on the visible absorption spectra of Wako lac dye and Thai lac dye. Wako and Thai lac dye solutions were freshly prepared prior to each determination. Wako lac dye (1.0364 g) was partially dissolved in deionized water, the mixture filtered, and the filtrate diluted to 500 mL with deionized water in a 500 mL volumetric flask. The Thai lac dye solution was prepared by treating the dye (0.5024 g) with deionized water (400 mL). This solution was filtered and the filtrate diluted to 500 mL with deionized water in a 500 mL volumetric flask. Into two series of twelve 10 mL volumetric flasks was added 0.50 mL of each lac dye solution. To the flasks, was added successively, 0.10, 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 4.00, 5.00, 6.00, 7.00, and 8.00 mL of aqueous 5×10^{-2} M $KAl(SO_4)_2{\cdot}12H_2O$ (alum). Each flask was then diluted to the mark with deionized water and the solutions allowed to stand for 30 min. After complex formation was complete, the absorption spectra and the changes in absorbance at 537 nm were recorded on a Cary 1E UV VIS spectrophotometer against deionized water as the blank and using quartz cells. The final concentrations of the alum solution in this experiment were as follows: 5.0×10^{-4}, 2.5×10^{-3}, 5.0×10^{-3}, 7.5×10^{-3}, 1.0×10^{-2}, 1.25×10^{-2}, 1.5×10^{-2}, 2.0×10^{-2}, 2.5×10^{-2}, 3.0×10^{-2}, 3.5×10^{-2}, and 4.0×10^{-2} M.

2.3.2.3. Nickel (II) ion solution studies on the visible absorption spectra of Wako lac dye and Thai lac dye. Lac dye solutions were prepared as noted in Section 2.3.2.2. Into each series of twelve 10 mL volumetric flasks was added 0.50 mL of lac dye solution. To the flasks, was then added consecutively, 0.10, 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 4.00, 5.00, 6.00, 7.00, and 8.00 mL of aqueous 5×10^{-2} M $Ni(NO_3)_2{\cdot}6H_2O$. Each flask was diluted to the mark with deionized water and the solutions then kept at room temperature for 30 min. After complex formation was complete, the absorption spectra and the changes in absorbance at 562 nm were recorded on a Cary 1E UV VIS spectrophotometer against deionized water as the blank and using quartz cells. The final concentrations of nickel (II) solution in this experiment were as follows: 5.0×10^{-4}, 2.5×10^{-3}, 5.0×10^{-3}, 7.5×10^{-3}, 1.0×10^{-2}, 1.25×10^{-2}, 1.5×10^{-2}, 2.0×10^{-2}, 2.5×10^{-2}, 3.0×10^{-2}, 3.5×10^{-2}, and 4.0×10^{-2} M.

2.3.3. Determination of the mole ratio for the nickel (II) ion laccaic acid A complex

The continuous variation (Job's) method was used to determine stoichiometry [15]. A series of aqueous solutions were prepared by mixing 5×10^{-4} M laccaic acid A and 5×10^{-4} M $Ni(NO_3)_2{\cdot}6H_2O$ and diluting to 5.00 mL with ethanol. The pH of each solution was measured, and the pH range observed was 2.8 4.2. The mixtures were allowed to stand at room temperature for at least 30 min and the changes in absorbance at λ_{max} of the longest wavelength absorption band were then measured.

2.4. Computational studies

Computational studies on the nickel (II) ion laccaic acid A complex were carried out on a Silicon Graphics Fuel processor running at 600 MHz with 1536 MB of random access memory using the Spartan '02 [16] program. Local energy conformations of the free acid were found using the AM1 technique [17] and resulting structures were used for the nickel (II) ion laccaic acid A complex minimum energy structure using the PM3 technique [18]. Non-binding sites in the octahedral coordination shell of the nickel (II) ion were filled by water molecules. In putative structures where different numbers of water molecules were required, additional water molecules were added at non-interacting distances to maintain constant mass.

3. Results and discussion

3.1. Effects of pH

Unsubstituted anthraquinone (9,10-anthraquinone) has a pale yellow colour and shows a weak absorption band at λ_{max} 405 nm ($\varepsilon = 60$) due to an $n \rightarrow \pi^*$ transition [19]. Thai lac dye contains at least three laccaic acids (A, B and C), which are all anthraquinone derivatives. These acids contain electron-donating substituents (hydroxyl groups) in the α and β positions that cause a significant bathochromic shift observed at λ_{max} 487 nm. They are thus typical donor acceptor systems in structure, with the carbonyl groups as the acceptors and the electron-releasing auxochromes as the donors [19,20].

The effect of pH on λ_{max} of the longest wavelength absorption band observed for Thai lac dye and Wako lac dye was very similar over the pH range 2.5 11. Over the pH range 2.5 6.0, there was only a small change in the λ_{max} value for the long wavelength maximum absorption in both dyes (λ_{max} 487 nm, pH 4.4 to λ_{max} 490 nm, pH 2.5). The oxygen atom of the quinone carbonyl groups in the 9- and 10-positions of the laccaic acid components of the dyes cannot be protonated [21] because of intramolecular hydrogen bond formation with the hydroxyl groups in the 1- and 4-positions, respectively, thus explaining the insensitivity to pH change in the acidic region. At high pH values, the phenolic and carboxylic acid groups in the anthraquinone dye components would be deprotonated. Resultant charge delocalization in the phenolate anions would lead to stabilization of the excited state with lowering of the energy of the transition, thus giving rise to a pronounced bathochromic shift (eg. λ_{max} 525 nm, pH = 9.9; Thai lac dye). This type of shift has been well documented with phenols [21,22].

3.2. Metal ion effects

The UV VIS spectra of Thai lac dye and Wako lac dye in the presence of metal ions are shown in Fig. 2. Significant changes occurred in

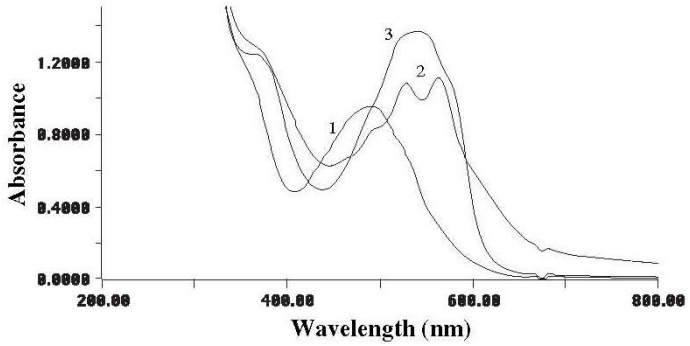

Fig. 2. Absorption spectra of Thai lac dye solutions: (1) Thai lac dye (200 ppm); (2) mixture of Thai lac dye (200 ppm) and nickel (II) ion (1×10^{-2} M); (3) mixture of Thai lac dye (200 ppm) and alum (1×10^{-2} M).

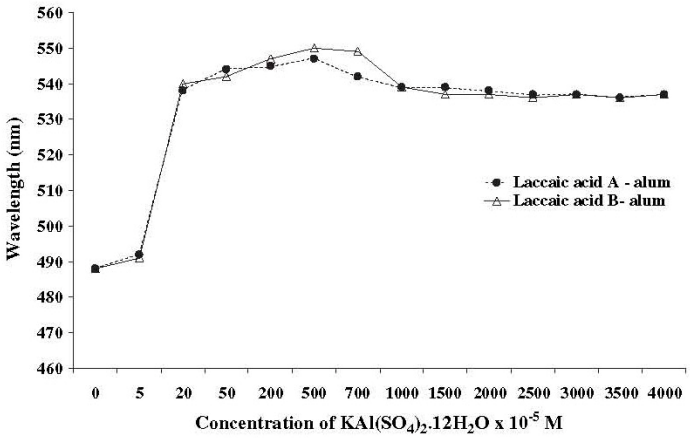

Fig. 3. The effect of alum concentrations on the most intense visible absorption band of laccaic acids A and B.

the bands absorbing at the longest wavelengths, and these changes were characteristic of the metal ion. With aluminum (III) ions, the λ_{max} value of the long wavelength absorption band was observed at 537 nm and with nickel (II) ions, two bands at λ_{max} values of approximately 523 and 562 nm were observed. In the absence of metal ions, Thai lac dye itself had a long wavelength absorption maximum at 487 nm.

Metal ions also caused a bathochromic shift of the long wavelength absorption bands of laccaic acids A and B themselves with increasing ion concentrations, for example with alum (Fig. 3). It was also observed that there was little change in pH (ca 4.0) over alum concentration ranges of 5.0×10^{-5} M to 4.0×10^{-2} M (Table 1). It is probable that the bathochromic shift observed with laccaic acids A and B, as with other dyes [20], occurs as a result of coordination by the lone pair electrons on the N or O donor atoms with the aluminum ion site, thus stabilizing the excited state relative to the ground state leading to longer wavelength absorption maxima [19,20] (Tables 2 and 3). In addition, an increase of the absorption intensity within the range of alum concentration was observed as shown in Table 4. This behaviour of laccaic acids is similar to the other dyes [20] where the increased absorption intensity plateaued at higher concentrations.

The effect of varying alum concentrations on Thai lac dye compared to Wako lac dye absorption spectra is presented in Fig. 4, and a similar bathochromic shift of the lac dye band was observed as for the laccaic acids. This result is consistent with these acids being the major components of the dyes. A greater absorbance difference between the dyes was observed on monitoring at λ 537 nm (Table 3). It is apparent

Table 1
Changes in pH of solutions of laccaic acids with alum and nickel (II) ion

Solution	Laccaic acid concentration (M)	Ion concentration (M)	pH
Laccaic acid A–alum	9.31×10^{-5}	5.0×10^{-5}–4.0×10^{-2}	4.45–3.90
Laccaic acid B–alum	1.05×10^{-4}	5.0×10^{-5}–4.0×10^{-2}	4.58–3.97
Laccaic acid A–Ni(II)	9.31×10^{-5}	5.0×10^{-5}–4.0×10^{-2}	4.90–4.44
Laccaic acid B–Ni(II)	1.05×10^{-4}	5.0×10^{-5}–4.0×10^{-2}	5.00–4.37

Table 2
Changes in pH of solutions of Wako lac dye and Thai lac dye with alum and nickel (II) ion

Solution	Lac dye concentration (ppm)	Ion concentration (M)	pH
Wako lac dye–alum	49	5.0×10^{-4}–4.0×10^{-2}	3.73–3.32
Thai lac dye–alum	50	5.0×10^{-5}–4.0×10^{-2}	4.10–3.37
Wako lac dye–Ni(II)	49	5.0×10^{-5}–4.0×10^{-2}	4.39–4.02
Thai lac dye–Ni(II)	50	5.0×10^{-5}–4.0×10^{-2}	5.63–5.12

that Thai lac dye, which was not as pure as Wako lac dye, absorbed less strongly. Furthermore, the pH of the Thai lac dye solution was consistently higher than Wako lac dye at all alum concentrations (Table 2).

The results of the study of nickel (II) ion effects on the visible spectra (λ_{max}) of laccaic acids A and B and the lac dyes are presented in Figs. 5 and 6 which indicated the larger bathochromic shift of laccaic acids A and B as the metal ion concentration increased. This behaviour was also observed in Wako lac dye and Thai lac dye. The higher metal ion concentration presumably results in stronger interaction with the dyes stabilizing the excited state and leading to a lower energy separation from the ground state. When the concentration of nickel (II) ions increased within the range of concentrations studied, the intensity of the long wavelength absorption band of laccaic acids A and B and Wako lac dye increased except in the case of Thai lac dye which was not much affected as shown in Tables 3 and 4. The different behaviour of Thai lac dye from laccaic acids A and B and Wako lac dye, was consistent with the lower purity of Thai lac dye hence the lower absorption. The similar pattern of behaviour of laccaic acids A and B and the lac dyes again indicated similar chromophores for these substances. These visible absorbance results differ in some respects from the interactions of other dyes with metal ions [20] where the increased absorption intensity plateaued at higher concentrations.

3.3. Nickel (II) laccaic acid A complex

The mole ratio of nickel (II):laccaic acid A as determined by Job's method (Fig. 7) was 1:1 and it

Table 3
Absorbance value dependence of Thai lac dye and Wako lac dye on concentrations of alum at $\lambda = 537$ nm and nickel (II) ion at $\lambda = 562$ nm

Alum concentrations $\times 10^{-4}$ (M)	Absorbance of Thai lac dye–alum	Absorbance of Wako lac dye–alum	Ni(II) ion concentrations $\times 10^{-4}$ (M)	Absorbance of Thai lac dye–Ni(II)	Absorbance of Wako lac dye–Ni(II)
0	0.1085	0.3068	0	0.0503	0.0633
5	0.2470	0.7192	5	0.2253	0.1968
25	0.2737	0.8502	25	0.2467	0.3626
50	0.2906	0.8966	50	0.2407	0.4451
75	0.2890	0.9064	75	0.2444	0.4933
100	0.2918	0.9412	100	0.2502	0.5041
125	0.2908	0.9479	125	0.2540	0.5404
150	0.3037	0.9548	150	0.2651	0.6035
200	0.2989	0.9421	200	0.2715	0.6870
250	0.3004	0.9917	250	0.2672	0.6837
300	0.3022	0.9726	300	0.2655	0.7694
350	0.3090	1.0245	350	0.2723	0.7521
400	0.3139	1.0382	400	0.2724	0.7373

Table 4
Absorbance value dependence of laccaic acids A and B on concentrations of alum at $\lambda = 537$ nm and nickel (II) ion at $\lambda = 562$ nm

Alum concentrations $\times 10^{-5}$ (M)	Absorbance of laccaic acid A–alum	Absorbance of laccaic acid B–alum	Ni(II) ion concentrations $\times 10^{-5}$ (M)	Absorbance of laccaic acid A–Ni(II)	Absorbance of laccaic acid B–Ni(II)
0	0.4621	0.4386	0	0.1257	0.1123
5	0.6526	0.6011	5	0.2022	0.1313
20	0.9887	1.0098	20	0.2598	0.2821
50	1.0025	1.1130	50	0.3866	0.2991
200	1.1789	1.2335	200	0.5222	0.5230
500	1.3032	1.3727	500	0.6424	0.7365
700	1.2960	1.4284	700	0.7620	0.7689
1000	1.3832	1.4615	1000	0.8140	0.8004
1500	1.4121	1.4547	1500	0.8655	0.8784
2000	1.4371	1.4916	2000	0.9090	1.0428
2500	1.4639	1.5357	2500	1.0254	0.9936
3000	1.4880	1.5156	3000	0.9979	1.0056
3500	1.5205	1.5034	3500	1.0526	1.0272
4000	1.4927	1.5391	4000	1.0771	1.0807

is likely that the rest of the coordination sites of nickel (II) ion would be occupied by solvent molecules. Computer-based molecular modelling studies using the Spartan '02 computer program (AM1/PM3; '02 Linux/Unix) were then undertaken to assess possible structures for this 1:1 nickel (II) laccaic acid A complex. In the modelling process, water was included as a ligand, and the phenolic and carboxylic acid groups were not ionized since in the experimental study the solution was in the pH range 2.8 4.2. The complex shown in Fig. 8 was the one with the lowest heat of formation (−195 kJ/mol) and it incorporated the quinone carbonyl oxygen and the phenolic group as ligands and four water ligands in the octahedral complex. Interestingly, the complex structure was also stabilized by hydrogen bonding interactions with the carboxylic acid functionality, and a phenolic group and the acetamide carbonyl group in the aryl substituent. These interactions, together

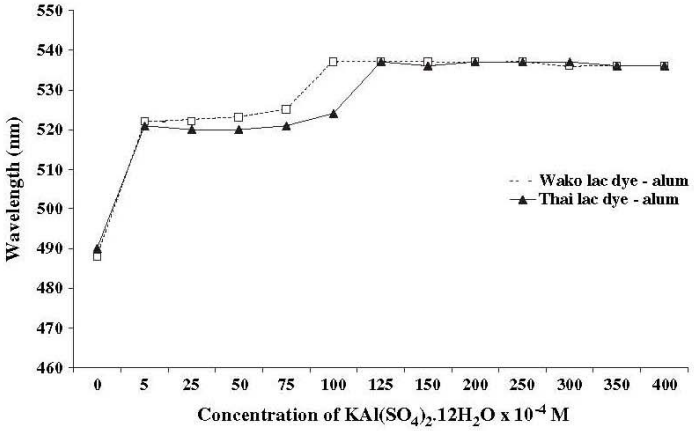

Fig. 4. The effect of alum concentrations on the most intense visible absorption band of Wako and Thai lac dye.

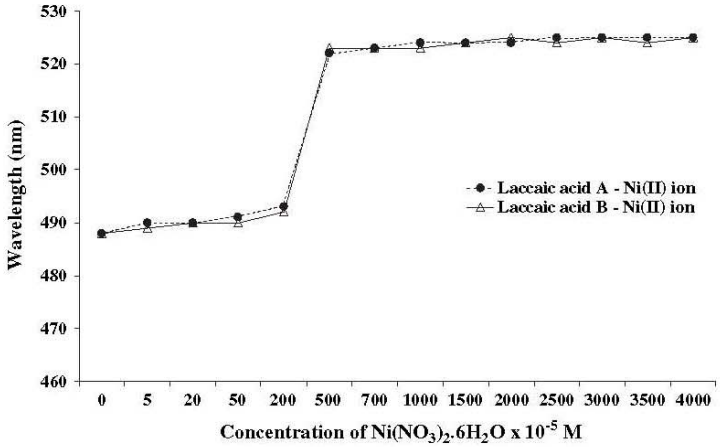

Fig. 5. The effect of nickel (II) ion concentrations on the visible absorption band of laccaic acids A and B.

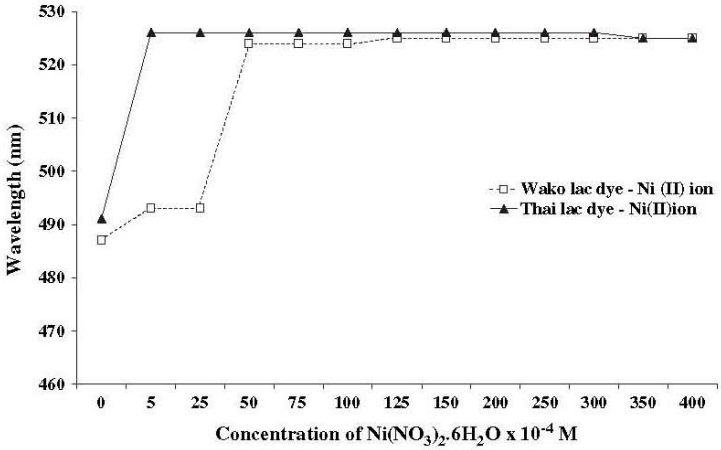

Fig. 6. The effect of nickel (II) ion concentrations on the visible absorption band of Wako lac dye and Thai lac dye.

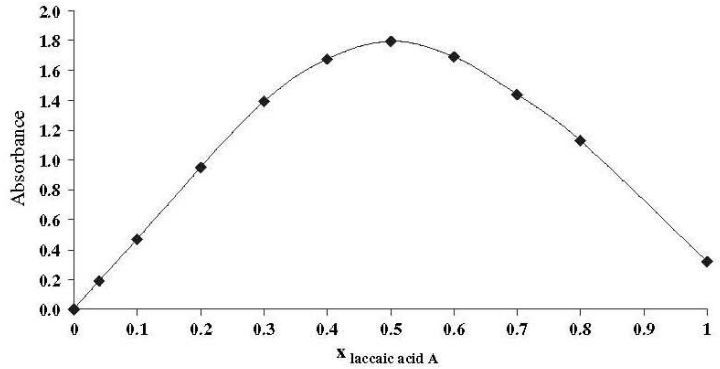

Fig. 7. Determination of the mole ratio for nickel (II) ion–laccaic acid A complex in ethanol.

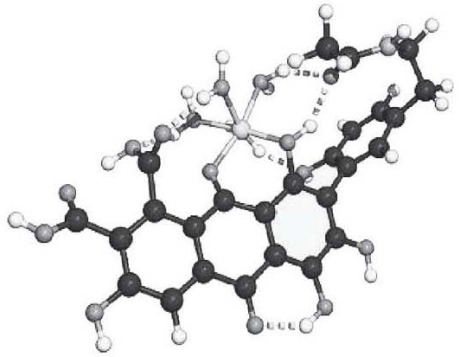

Fig. 8. The proposed structure of nickel (II) ion–laccaic acid A complex from computer-based modelling (Spartan '02 Linux/ Unix; PM3).

with increased electron density at the quinone carbonyl oxygen from lone pair electron delocalization from the phenolic groups at positions 3 and 6, presumably account for this preference for nickel ion complex formation at the position proposed. In support of the latter point, an electrostatic potential map (Spartan program, AM1) of laccaic acid A confirmed higher negative potential at the 9-quinone carbonyl oxygen group compared with the potential at the other quinone group.

4. Conclusions

When the pH of the lac dye solution was increased from 2.5 to 11, a substantial bathochromic shift of the lac dye visible absorption band was observed in both Wako and Thai lac dyes. Also increasing the metal ion concentration, for both alum and nickel (II) ion, caused a bathochromic shift of the visible absorption bands in both dyes (and in their laccaic acid components, laccaic acids A and B). The bathochromic shifts observed are consistent with the lone pair electrons in the donor atoms (N and O in the lac dyes/laccaic acids) participating in metal ion coordination and stabilizing the excited state relative to the ground state. Support for this participation was obtained from a modelling study on the proposed nickel (II) laccaic acid A complex.

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