ADSORPTION OF SELECTED DYES BY CHEMICALLY MODIFIED *Uncaria gambir* EXTRACT

by

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Thesis submitted in fulfilment of the requirements for the degree of Master of Science

UNIVERSITI SAINS MALAYSIA
2012

ACKNOWLEDGEMENTS

Alhamdulillah. I am so grateful to Allah SWT for giving me opportunity to conduct and to complete this research. I would like to acknowledge my deepest gratitude to my supervisor, Prof. Dr. Mohd Jain Noordin Mohd Kassim for the ideas, suggestions and guiding during conducting this research. I am also like to thank my beloved parents, Mr. Achmad Helza and Mrs. Khatijah Jusoh and also my siblings for their constant support and encouragement.

A grateful thanks to Prof. Mohd Jain Noordin research group, Mr. Tong Kim Suan, Ms. Rozaini Che Amat, Ms. Tan Lean Seey, Mr. Hazwan Hussin, Ms. Nur Hazwani Dahon, Ms. Nurul Nu'Aim Razali, Mr. Tan Kang Wei, Ms. Nurul Azwin Usol Ghafli, Mr. Hairul Safley Hamdan, and Ms. Nurul Izni Kairi. This research complete and successful with the participation and kindly helps of these persons.

I wish to convey my gratitude to School of Chemical Sciences, USM for providing all the facilities and not forgetting to all staff and friends of the school. I am also appreciating and thank the Institute of Graduate Studies and Universiti Sains Malaysia for the Research University Postgraduate Research Grant Scheme and Universiti Sains Malaysia Fellowship.

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LIST OF ABBREVIATION AND SYMBOLS

ΔG^o	: Standard free energy change (kJ mol^{-1})
ΔH^o	: Standard enthalpy change (kJ mol^{-1})
ΔS^o	: Standard entropy change (J mol^{-1} K^{-1})
ΔpH	: Different between pH initial and pH final
1/n	: Heterogeneity factor
BET	: Brunauer, Emmet and Teller
BJH	: Barret, Joyner and Halenda
C	: The intercept of the line which is proportional to the boundary layer thickness
CAE	: Catechin equivalent
C_i	: Initial dye Concentration (mg L^{-1})
C_e	: Concentration of dye at equilibrium (mg L^{-1})
C_a	: Concentration of dye adsorbed (mg L^{-1})
C_d	: Concentration of dye desorbed (mg L^{-1})
DR 23	: Direct Red 23 dye
E	: Mean energy adsorption
FTIR	: Fourier transform infrared spectroscopy
FW	: Formula weight
h	: The initial adsorption rate (mg g^{-1} min^{-1})
k_1	: The rate constant of pseudo-first-order (min^{-1})
k_2	: The rate constant of pseudo-second-order (g mg^{-1} min^{-1})
K_L	: Langmuir constants related to the adsorption energy (L mg^{-1})
K_F	: Freundlich constant related to the adsorption capacity (mg g^{-1})
K_p	: Intraparticle diffusion constant (mg g^{-1} $min^{-0.5}$)
MB	: Methylene Blue dye
MGA	: Modified gambir adsorbent
pH_{zpc}	: pH zero point of charge
P/P_0	: Relative Pressure
q_e	: Equilibrium adsorption capacity (mg g^{-1})
q_m	: Maximum adsorption capacity (mg g^{-1})

q_t : Adsorption capacity at time, t (mg g^{-1})

$q_{e,cal}$: Calculated equilibrium adsorption capacity (mg g^{-1})

$q_{e,exp}$: Experimental equilibrium adsorption capacity (mg g^{-1})

R_L : Dimensionless separation factor

R^2 : Correlation coefficient

S_{BET} : BET surface area (m^2 g^{-1})

SY : Sunset Yellow FCF dye

t : Time (min)

UV-Vis : Ultraviolet and Visible Absorption Spectroscopy

V_{tot} : Total pore volume (cm^3 g^{-1})

V_{mic} : Micropore volume (cm^3 g^{-1})

V_{meso} : Mesopore volume (cm^3 g^{-1})

ε : Polanyi potential

PENJERAPAN PEWARNA TERPILIH OLEH EKSTRAK *Uncaria gambir* TERUBAHSUAI SECARA KIMIA

ABSTRAK

Ekstrak *Uncaria gambir* diubahsuai secara kimia untuk menghasilkan zat penjerap yang baru untuk penjerapan metilena biru (MB), pewarna sunset kuning fcf (SY), dan pewarna langsung merah 23 (DR 23) daripada larutan akueus. Ekstrak etil asetat gambir didapati mengandungi kandungan fenolik dan flavonoid yang tertinggi. Ekstrak ini menjalani prarawatan dengan formaldehid dan asid hidroklorik untuk menghasilkan penjerap gambir terubahsuai (MGA) yang dicirikan menggunakan analisis spektroskopi inframerah Fourier (FTIR), analisis mikroskop elektron pengimbas (SEM) dengan serakan tenaga sinar-X (EDX), pengukuran luas permukaan Brunauer-Emmett-Teller (BET), dan nilai pH titik sifar cas (pH_{ZPC}). Kajian penjerapan berkelompok di bawah pelbagai keadaan dijalankan di mana kesan pH, dos penjerap, masa penjerapan, dan kepekatan awal pewarna dikaji. Data penjerapan untuk MB, SY dan DR 23 didapati sepadan dengan isoterma Langmuir dengan kapasiti penjerapan maksimum pada suhu 303 K masing-masing sebanyak 149.3 mg g^{-1}, 6.4 mg g^{-1} dan 26.3 mg g^{-1}. Proses penjerapan bagi semua pewarna mengikuti model kinetik pseudo-tertib-kedua. Data kajian termodinamik mencadangkan bahawa proses penjerapan MB, SY dan DR 23 oleh MGA adalah endotermik dan spontan. Penyaherapan maksimum pewarna MB diperolehi dalam larutan akueus pada pH 2 manakala bagi SY dan DR23 telah dicapai dalam larutan akueus pada pH 10. Proses penjerapan MB, SY dan DR 23 oleh MGA didapati berlaku secara kimia dan fizikal berdasarkan data isoterma, termodinamik dan

penyaherapan. Keputusan menunjukkan bahawa ekstrak *Uncaria gambir* mempunyai potensi untuk dijadikan sebagai penjerap bagi penyingkiran pewarna daripada larutan akueus.

ADSORPTION OF SELECTED DYES BY CHEMICALLY MODIFIED *Uncaria gambir* EXTRACT

ABSTRACT

Uncaria gambir extract was chemically modified to develop a novel adsorbent for the adsorption of Methylene Blue (MB), Sunset Yellow FCF (SY), and Direct Red 23 (DR 23) from aqueous solution. It was revealed that the ethyl acetate extraction of gambir showed highest total phenolic and total flavonoid contents. The gambir extract was subjected to pretreatment with formaldehyde and hydrochloric acid to produce the modified gambir adsorbent (MGA) which was then characterized by Fourier transform infrared (FTIR) spectroscopy, scanning electron microscope (SEM) with energy dispersive X-ray (EDX) spectroscopy, Brunauer-Emmett-Teller (BET) surface area, and pH zero point of charge (pH_{zpc}). Adsorption tests were conducted in batch reactors under various conditions where the effect of pH, adsorbent dosage, contact time, and dye initial concentration were studied. The adsorption experimental data of MB, SY and DR 23 were well described by the Langmuir isotherm and the maximum adsorption capacity at 303 K was found to be 149.3 mg g^{-1}, 6.4 mg g^{-1} and 26.3 mg g^{-1}, respectively. The adsorption process for all dyes was followed by pseudo-second order kinetic model. Thermodynamic study had showed that the adsorption process of MB, SY and DR 23 onto MGA were endothermic and spontaneous. Maximum desorption study of MB was obtained in pH 2 aqueous solution meanwhile for SY and DR 23 were achieved in pH 10 aqueous solution. Based on the isotherm, thermodynamic and desorption studies, the adsorption of MB, SY and DR 23 onto MGA was chemical and physical adsorption

processes. The research indicated that *Uncaria gambir* extract has a potential to be employed as an adsorbent for the removal of dyes from aqueous solutions.

CHAPTER 1

INTRODUCTION

1.1 ENVIROMENT POLLUTION

In the literature stated by Inglezakis and Poulopoulos (2006a), various pollutants had been discharged into environment nature especially into aquatic environment due to human activities. These human activities threaten not only the fresh water supply but also marine life. Although water covers 71 % of the planet's surface, but almost 97.5 % of the total is the saline water of oceans which is not suitable for drinking, watering, or industrial use. The remaining 2.5 % is fresh water. Therefore, the hydrological cycle in nature will purified and reallocated the fresh water to be consumed by human. However, these natural cycle also become inadequate due to pollution. Simultaneously, human activities also produce and release large amount of wastewater into the environment. Hence, water pollution and contamination will occurred due to this situation. This situation is expected to be worst in the near future, especially in high populated, agricultural and industrial areas.

Water pollution can be defined as any physical, chemical, or biological alteration in water quality that effected living organisms. The sources of water pollution are divided into point sources and nonpoint sources. Chemical industries and human communities are the example of point sources which are mainly caused the pollution of surface waters like rivers, lakes and seas. Nonpoint sources of water pollution like agricultural activities and landfill leakages are mainly contributed by the groundwater resources. However, these point sources can be treated with wastewater

treatment meanwhile nonpoint sources can only be minimized (Inglezakis and Poulopoulos, 2006b).

A common contaminant found in wastewater is dyes (Forgacsa et al., 2004). Dyes are applied in textile manufacturing, leather tanning, paper production and food technology industries. Choy et al., (1999), reported that the total dyes consumption of the textile industry alone is in excess of 107 kg $year^{-1}$ and is estimated 90 % of this total ends up on fabrics. Consequently, approximately 106 kg $year^{-1}$ of dyes are discharged into waste streams by the textile industry. Dyeing wastewater discharged to natural receiving waters may bring unacceptable for public consumption (Inthorn et al., 2004). Therefore, the wastewater treatment is desirable to overcome this problem.

1.2 DYES

It is estimated that about 40,000 tonnes of dyes are not used but are discharged into wastewaters. This is out of a total production of about 450,000 tonnes. Dyes are broadly classified as anionic, cationic and non-ionic depending on the ionic charge on the dye molecules (El-Sayed, 2011). Acid, direct and reactive dyes are namely in the anionic dyes group meanwhile basic and dispersive dyes are characterized as cationic and non-ionic dyes, respectively. Dyes are generally required to dissolve in water because dyes are almost invariably applied to the textile materials from an aqueous medium.

Dyes are highly visible. Therefore, the appearance of dyes can be seen even minor of it had been released into environment especially in water. The presence of dyes in natural streams can cause serious harm to the aquatic life by increasing toxicity and chemical oxygen demand, and by hindering photosynthetic phenomena through reduction of light penetration (Oliveira et al., 2008). Many of dyes wastes are classified as toxic and carcinogenic compound (Vandevivere et al., 1998). A few dyes lead to some significant toxic effects. Acute toxic is the effect due to short-term exposure to a substance meanwhile chronic toxic refers to the effect of regular exposure over a prolonged period of time. Certain dyes which contain azo groups are carcinogenic due to reductive cleavage of the group to give aromatic amines.

Methylene Blue is a kind of cationic dye (basic dye) contained of heterocyclic aromatic chemical compound with the molecular formula $C_{16}H_{18}N_3SCl$. The IUPAC name of Methylene Blue is 3,7-bis-(dimethylamino)-phenothiazin-5-ium chloride. This dye is generally classified as a basic dye and cationic species due to the presence of positively charged quaternary nitrogen atoms (as $–NR_3^+$, or $= NR_2^+$). These groups enhance solubility of dye in water due to their ionic character (Christie, 2001a). At room temperature it appears as a solid, odorless, dark green powder and yields a blue solution when dissolved in water. Figure 1.1 shows the structure of Methylene Blue.

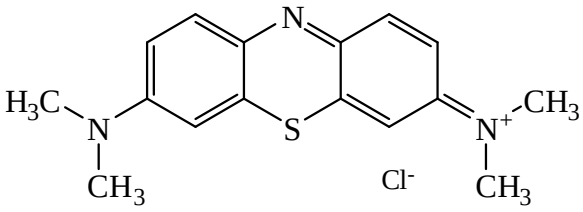

Figure 1.1: Methylene Blue structure.

Sunset Yellow FCF is also known as Orange Yellow S. It is anionic dye (acidic dye) with IUPAC name disodium 6-hydroxy-5-(4-sulfonatophenylazo)-2-napthalene-sulfonate. It exists as an orange red powder and present as yellow orange colour in the aqueous form. The formula of Sunset Yellow is $C_{16}H_{10}N_2Na_2O_7S$. Most yellow, orange and red colour dyes belong to the azo chemical classes due to the presence of azo (N=N) group in their molecular structure. Acid dyes commonly contain one or more sulfonate ($-SO_3^-$) groups, usually as sodium (Na^+) salts. These groups enhance water solubility for the dye and ensure the dye carrying a negative charge as anionic (Christie, 2001b). Figure 1.2 shows Sunset Yellow FCF structure.

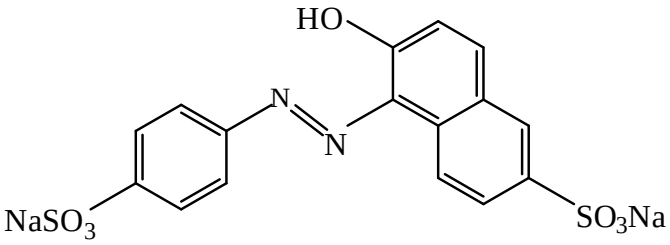

Figure 1.2: Sunset Yellow FCF structure.

The IUPAC name for Direct Red 23 is known as disodium 3-[(4-acetamidophenyl)azo]-4-hydroxy-7-[[[[5-hydroxy-6-(phenylazo)-7-sulphonato-2-naphthyl]amino]carbonyl]amino]naphthalene-2-sulphonate. Its molecular formula is $C_{35}H_{25}N_7Na_2O_{10}S_2$ and it exists as a purple red powder at room temperature. Direct dyes are an anionic dye and the structures are similar to acidic dyes due to the presence of sulfonate ($-SO_3^-$) and azo (N=N) groups. However, direct dyes have different application properties because of their molecular size and shape. Generally, direct dyes have large molecules and long shapes. Figure 1.3 shows the structure of Direct Red 23 dye.

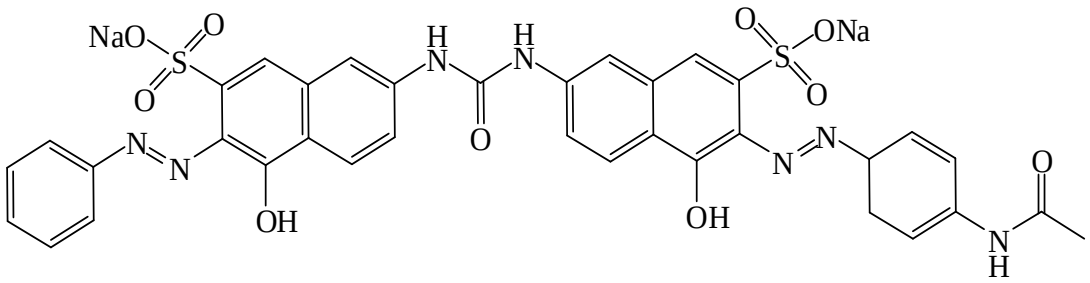

Figure 1.3: Direct Red 23 structure.

The problem of dyes effluent has attracted the critical attention of public and authorities. It is rather difficult to treat dye effluents because of their synthetic origins and their mainly aromatic structures, which are biologically non-degradable (El-Sayed, 2011; Hameed et al., 2007a). According to Dursun et al., (2007), The complex aromatic structures of dyes make them more stable and more difficult to remove from the effluents discharged into water bodies. Thus, a lot of alternative treatment methods had been approached to remove these dyes effluents.

1.3 WASTEWATER TREATMENT METHOD

Dyes are usually stable to photo-degradation, bio-degradation and oxidizing agents which has led to intensive investigations on physical or chemical methods to remove color from textile effluent (Ahmed et al., 2009; Ramakrishna and Viraraghavan, 1997a). Chemical treatments method is one of the dyes removal methods involved oxidative degradation by using chlorine or ozone. Even though, this ozone treatment is effective, it is still expensive. Physical treatments like adsorptions of dyes onto substrates such as activated carbon, silica, or ion exchange resin is also effective for dyes removal. Among these methods, adsorption has been shown to be an effective with its efficiency, capacity and applicability on a large scale to remove dyes as well as having the potential for regeneration, recovery and recycling of adsorbents (Choy et al., 2004, Dabrowski, 2001, Chern and Wu, 2001, Robinson et al., 2001).

1.3.1 Adsorption

Skoulikides (1989), stated that the term "sorption" is used to describe every type of capture of a substance from the external surface of solids, liquids, or mesomorphs as well as from the internal surface of porous solid or liquids. Adsorption is an attachment of particles to a surface (Atkins and Paula, 2002). Meanwhile, according to Ocsik (1982), adsorption refers to changing in the concentration of molecules (atoms, ions) at the surface, while absorption consists of the penetration of a substance from one phase into the bulk of another by diffusion process. Adsorbate is the substance that adsorbs meanwhile adsorbent is the underlying material.

Adsorption types are depend on the bonding involved between the adsorbate molecules and the atom which compose the adsorbent surface (Koh, 2006).

Adsorption can be classified as physical sorption (physisorption) and chemical sorption (chemisorption). According to Inglezakis and Poulopoulos (2006c), the electron exchange process is absent in physisorption but intermolecular attaractions between favourable energy sites take place and independent of the electronic properties of the molecules involved. The adsorbate is attached to the surface by a weak van der Waals forces and multiple layers may be formed. Whereas in chemisorption, an exchange of electrons between specific surface sites and solute molecules is involved and as a result chemical bonding is formed. Generally, in chemisoption only a single layer can be formed. Oscik (1982), stated that the type of phases in contact for adsorption process can be classified into four systems which are solid-liquid, solid-gas, liquid-liquid and liquid-gas. The nature of the adsorbate and adsorbent surface such as surface area and porosity will affect the adsorption process.

Figure 1.4 shows the solid-solute adsorption system. The four main classes of isotherm are L (Langmuir), H (high affinity), S and C (constant position) (Giles et al.,1974a). The shape of the isotherms at higher concentrations for each classes will respectively divided into several subgroups such as subgroup 1,2,3,4, and 5. Besides, the adsorption mechanism, orientation of adsorbed solute etc also can be determined based on the shape of the isotherms L, H, S and C. The L-type isotherm reflects a relatively high affinity between the solid and the solute meanwhile the H-type isotherm indicates a very strong solid-solute interaction. Both isotherms often indicate chemical adsorption. The S-type isotherm represents stronger interaction

between solid-solute than solid-solid. The C-type isotherm represents a constant relative affinity of solute for the solid and usually observe at low range of adsorption. At high adsorption level, deviation from the linear isotherm might happen (Megat, 2010).

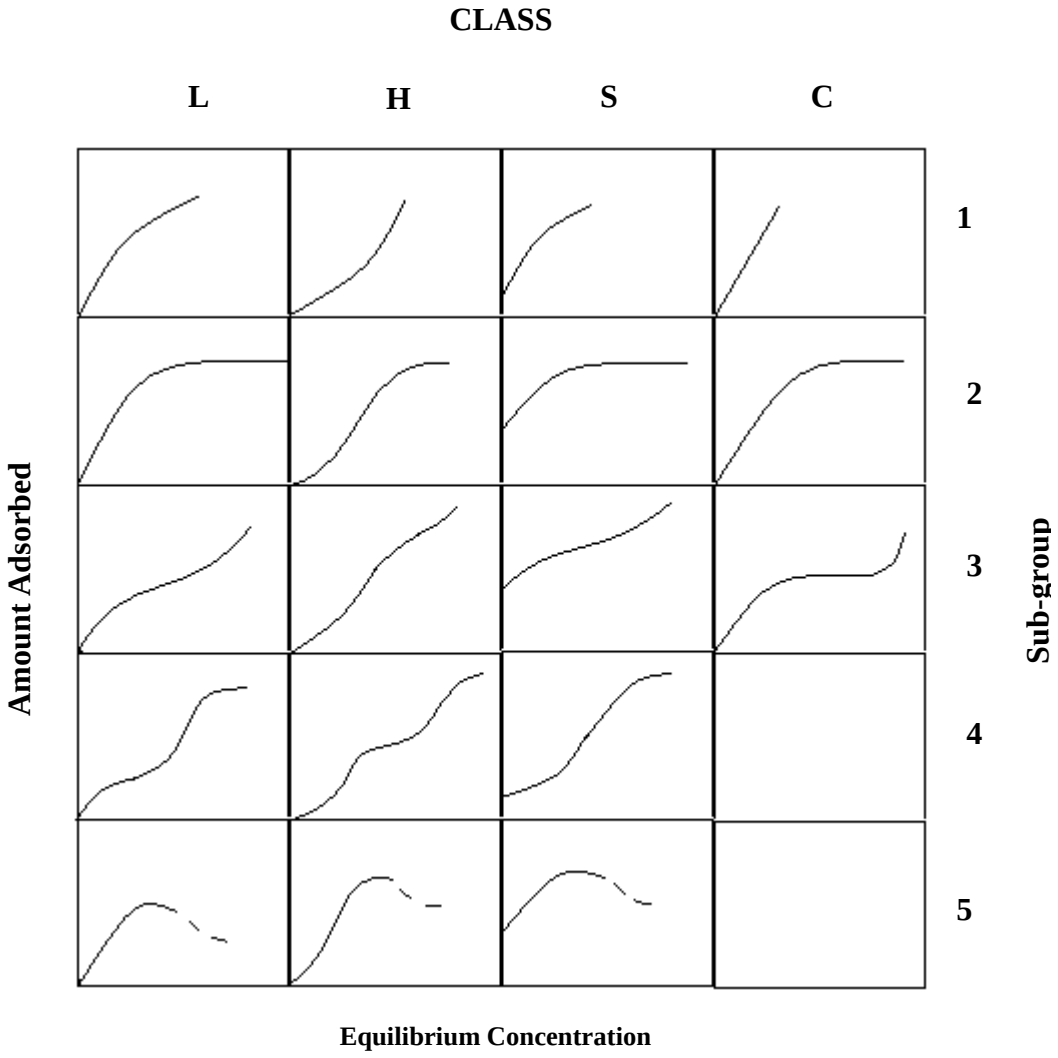

Figure 1.4: Solid solute adsorption (SSA) isotherm system (Giles et al.,1974a).

According to Giles et al., (1974a; 1974b), the subgroups respectively represents the layer formation in solid-solute adsorption. In subgroup 1, the adsorbate monolayer has not been completed. Subgroup 2 shows the completion of the first monolayer. Subgroup 3, 4 and 5 represents the formation of second layer and complete formation of second group. The L-type isotherm shows that the solute (adsorbates) lies flat on the solid (adsorbents) surface. The H-type isotherm corresponds to the adsorption of very large adsorbates like polymers or adsorbable micelles. In general, the H-type and L-types are typical shapes for chemisorptions. The S-type isotherm gives a vertical orientation of the adsorbate on the adsorbents surfaces. The C-type isotherm is normally shown by adsorption of nonionic dyes, amino acids and peptides on microporous inorganic solids such as clays. Thus, the S-type and C-type are more favorable to physical adsorption (Megat, 2010).

There are many environmental practical applications of adsorption and many others are being developed (Noble and Terry, 2004). Adsorption can be employed in wastewater treatment since it gives a lot of advantages. For instance, it gives high removal efficiency, enable the removal of refractory or toxic organic compounds, simple installation and maintenance, capability for fully automatic operation and available for large and variety of adsorbents (Inglezakis and Poulopoulos, 2006d).

1.3.2 ADSORBENT

Adsorption is one of the methods used in wastewater treatment since it is economical, effective and simple to design. However, the adsorption process is influenced by the nature of the adsorbate and its substituent groups. The presence and concentration of surface functional groups plays an important role in the adsorption capacity and the removal mechanism of the adsorbate (Lahaye, 1998; Yenisoy-Karakas et al., 2004). There are several types of adsorbent being applied in industrial wastewater treatment such as activated carbon, silica gel, and alumina. Activated carbon can be produced from carbonaceous material including coal like lignite, peat, wood, nutshells and coconut.

Activated carbon has the advantage of exhibiting a high adsorption capacity for color pollutants due to their large surface area and high porosity structures (Dursun et al., 2007). Although, activated carbon is more effective and efficient compared to other adsorbents, its application is restricted due to high cost and it is hardly decomposed which may causes numerous environmental problems. Therefore, an alternative of low cost and eco-friendly adsorbents had been intensively investigated by researchers. According to Nasuha et al., (2010), agro-waste adsorbent gives more advantages such as low cost, high efficiency, minimization of chemical or biological sludge, no additional nutrient requirement, and regeneration of adsorbent and possibility of effluent recovery. Table 1.1 shows the summary of basic, acidic and direct dyes adsorption onto various types of agro-waste adsorbents.

Table 1.1: Summary of basic, acidic and direct dyes adsorption onto various types of agro-waste adsorbents.

Dyes	Adsorbent	q_m (mg g^{-1})	Reference
Basic Dye			
Methylene Blue	Rejected tea	147.0	Nasuha et al., 2010
	Coffee ground	18.7	Franca et al., 2009
	Garlic peel	82.6	Hameed et al., 2009
	Wheat bran carbon	122.0	Ozer and Dursun, 2007
	Coir pith carbon	5.9	Kavita and Namasivayam, 2007
	Mangrove bark	178.6	Jain et al., 2008
	Tea waste	85.2	Tamez et al., 2009
	Peanut hull	123.5	Dursun et al., 2007
	Mango seed kernel	142.9	Vasanth and Kumaran, 2005
	Rattan sawdust	294.1	Hameed et al., 2007a
Acid Dye			
Acid Black 1	Fly ash	10.3	Deshuai et al., 2010
Acid Blue 193	Fly ash	10.9	Deshuai et al., 2010
Acid Blue 92	Egg shell membrane	0.5	Arami et al., 2008
Acid Red 14	Egg shell membrane	1.0	Arami et al., 2008
Acid Green 25	Palm ash	123.4	Hameed et al., 2007b
Sunset Yellow FCF	Powdered peanut Hull	13.9	Gong et al., 2005
Fast green FCF	Powdered peanut hull	15.6	Gong et al., 2005

Table 1.1 (continue)

Dyes	**Adsorbent**	**q_m (mg g^{-1})**	**Reference**
Direct Dye			
Direct blue 71	Palm ash	400.0	Ahmad et al., 2007
	Wheat shell	40.8	Bulut et al., 2007
Direct blue 1	Native *Trameters versicolor*	101.1	Bayramoglu and Arica, 2007
Direct red 128	Native *Trameters versicolor*	189.7	Bayramoglu and Arica, 2007
Direct red 80	Orange peel	21.1	Doulati et al., 2007
Direct red 12B	Coconut coir pith	76.3	Sureshkumar and Namasivayam, 2008
Direct red 23	Mangrove bark	21.6	Tan et al., 2010
	Rice husk	4.4	Ahmed et al., 2005
	Orange peel	10.7	Arami et al., 2005

1.4 *Uncaria gambir*

Uncaria gambir is also known as Gambir, Gou Teng, Asen'yaku, Cat's Claw, Una de Gato, and Pale Catechu (Taniguchi et al., 2007a; Remington and Wood, 1918; Chong, 2009). It is a member of the *Rubiaceae* family (Heitzman et al., 2005). This species are widely distributed in tropical regions, such as Malaysia, Singapore and Indonesia. Cultivation of gambir varies based on the species and region (Risdale, 1978). According to Tong (2009), the gambir plant is able to grow until about eight feet high and has oval shape of leave around 8 to 14 cm in length with 4 to 5 pairs or nerves.

Moreover, each pair of leaves may have a pair of globular inflorescences and the gambir flowers also originate at the base of the leaves. Gambir plant can only be grown only at certain condition, such as the pH of soil uses for gambir plantation must be within the pH range from 4.8 to 5.5. Besides that, the plant must be grown at 200 to 800 meter above sea level with rainfall around ±3.3 mm per year and humidity around 70 to 85 % (Hadad et al., 2009). Figure 1.5 and Figure 1.6 show the *Uncaria gambir* plant and *Uncaria gambir* cubes, respectively.

Figure 1.5: *Uncaria gambir* plant (Raintree, 1996).

Figure 1.6: *Uncaria gambir* cubes (Gambir, 2010).

Freudenberg and Purrmann (1923), has isolated two active polyphenol compounds from dried aqueous extract of gambir which is (±)-catechin and (+)-epicatechin. The isolation of seven new biflavonoids which is gambiriin A1, A2, A3, B1, B2, B3 and C from gambir has been done with further works by Nonaka and Nishioka (1980). The seven new biflavonoids were characterized using mass spectrum (Nonaka and Nishioka, 1980), ^{1}H and ^{13}C NMR (Taniguchi et al., 2007b), reverse phase-high performance liquid chromatography, RP-HPLC and gel permeation chromatography, GPC (Taniguchi et al., 2007a). Further works done by Taniguchi et al., (2007a), was the quantitative analysis of gambir which shown that the total flavan content was ranged from 24% to 79 % by using the vanillin-acid estimation method while the analysis by using RP-HPLC techniques gives around 76 % of catechin content, 1.5 % of epicatechin content and 1 % each for the content of gambiriin B1, B3 and A1. The RP-HPLC analysis indicated that the catechin compound is the highest constituent in gambir.

Besides, Hayani (2003) and Idris (2007), reported that gambir also contains a few amount of quercetin that behave as colorings agent, which makes the gambir extract appears in yellow colour. According to Hayani (2003), the catechin content in the gambir extract ion by using a hot plate shows higher percentage of catechin content with the range around 81 % to 88 %. Chang et al., (2002), reported that *Uncaria* species has many general traditional medicinal uses such as treatments for wounds and ulcers, fevers, headaches, gastrointestinal illnesses, and bacterial/fungal infections. Moreover, a tanning material was also obtained from the aqueous extract of *Uncaria gambir* leaves and stems (Phillipson et al., 1978; Ahmed et al., 1978). Recently years, the biological activities of extracts and pure compounds isolated

from *Uncaria* species have been increasingly investigated. These include anticonvulsive, antiinflammatory, antimutagenic, antioxidant, cytoprotective, hypotensive, and immunoregulatory effects (Laus, 2004). The anti-oxidative properties of catechin in gambir have attracted researchers to study deeper on its applications.

According to Hayani (2003), Remington and Wood (1918), and Zamarel and Risfaheri (1991), traditionally gambir has been used for skin tanning, colouring in textile and chewing. Furthermore, gambir is also often used as remedies for diarrhea and sore throat (Taniguchi et al., 2007b). A research done by Pambayun et al., (2007), reported that gambir extract gave highest inhibition effect on the Gram-positive bacteria such as *Streptococcus mutans*, *Staphylococcus aureus* and *Bacillus subtilis*. Nasrun et al., (1997) and Idris (2007), stated that gambir also exhibits the plant pesticide properties by inhibiting the growth of *Phytophthora cinnamomi* fungi in cinnamon plant and preventing leave spot disease that caused by *Fusarium sp* in *Citronella crop* leaves. Moreover, Hazwan and Jain (2010), have reported the potential of gambir extract as mild steel corrosion inhibitor.

1.5 Catechin

According to Cheong et al., (2005), the structure of flavonoids is the C6-C3-C6 skeletal where two phenyl rings are connected via a unit composed of three. In green plants, flavonoids are present in roots, stalks, leaves, fruits, etc. The plant body will be protected from ultraviolet lights and bacteria by flavonoids (Robards and

Antolovich, 1997). Besides that, flavanoids classified as polyphenolic compounds due to the attachment of two or more hydroxyl groups to each phenyl ring. It was reported that some health-enhancing effects such as anti-tumor and/or anti-cancer activities (Jang et al., 1997; Middleton, 1996), antibacterial (Singleton and Esau, 1969), and antioxidant (Afanas'ev et al., 1989; Mora et al., 1990) are known in various types of natural foods because of the present of polyphenolic compounds.

A few types of flavonoids like flavonone, flavone, flavonol, catechin, epicatechin, etc are origin from the "chalcone" which is the first flavonoid that form in the plant body (Markham, 1981). These compounds can be found in most plant body like in teas (Row and Jin, 2006; Cheong et al., 2005), sea buckthorn (Zu et al., 2006) and others. However, the flavanoids are easier to be stored in the aqueous space of the plant cell by existed as glycosides. Glycosides are chemically more stable and more soluble in water (Cheong et al., 2005).

Catechin is a polyphenolic compound of flavonoid group (Ramos-Tejada et al., 2002). It is snow-white in colour with a silky appearance, crystallisable in fine needles and melting at 175 ºC to 177 ºC (Catechin, 2008). According to Hazwan (2010), catechin is slightly soluble in cold water with which it soften and swells up and completely soluble in boiling water which deposits on cooling. It is also completely soluble in alcohol or ether. The chemical structure of catechin (flavan-3-ol) molecule is shown in Figure 1.7.

Catechin is a non-planar molecule because it possesses four –OH phenolic group that is located in two benzene rings connected via a non aromatic ring in which oxygen atom (C-O-C group) and one more –OH group are present (Ramos-Tejada et al., 2002). The –OH phenolic in the first ring are in meta-position and quinines cannot be formed. Unlike the first ring, the –OH phenolic in the second ring are in the ortho-position in which they can react to quinines. According to Friedman and Jurgens (2000), a chemical reaction will depend on –OH and π-electron system. Thus, the spatial arrangement of ployphenol molecules is important for their stability against pH.

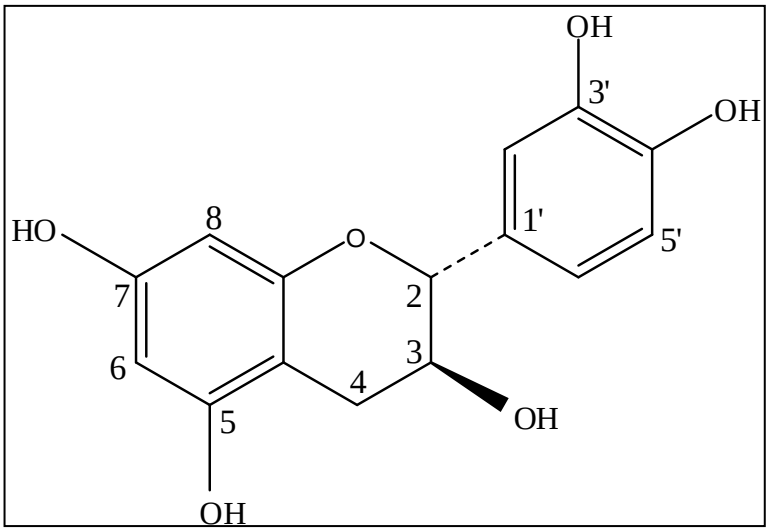

Figure 1.7: The chemical structure of catechin.

1.6 Research Objectives

The aim of this research is to study the potential of modified *Uncaria gambir* adsorbent for the adsorption of dyes which are Methylene Blue, Sunset Yellow FCF and Direct Red 23. The objectives of this research are as follows:

1. To determine the total phenol content and total flavonoid content of gambir extract.
2. To produce and to characterize the modified gambir adsorbent (MGA).
3. To study the effects of parameters such as pH, adsorbent dosage, contact time and initial concentration of dyes adsorption on modified gambir adsorbent.
4. To evaluate the adsorption data with isotherm and kinetic models.
5. To determine the thermodynamic parameters such as enthalpy change (ΔH^o), free energy change (ΔG^o) and entropy change (ΔS^o) at different temperature.

CHAPTER 2

MATERIALS AND METHODS

2.1 Chemicals and Instruments

2.1.1 Chemicals

1. Acetone (99.5 %) – QRec (New Zealand)
2. Aluminium Chloride (98.5 %) – SYSTERM (Selangor , Malaysia)
3. (+)- Catechin Hydrate (98.0 %) – SIGMA ALDRICH (Selangor, Malaysia)
4. Direct Red 23 (30 %) - ALDRICH (Selangor, Malaysia)
5. Ethyl acetate (99.5 %) – QRec (New Zealand)
6. Folin-Ciocalteu Reagent (2N) - SIGMA ALDRICH (Selangor, Malaysia)
7. Formaldehyde (37 %) – QRec (New Zealand)
8. Gallic acid (98.0 %) – Fluka (New York, United States)
9. Hydrochloric acid (37 %) – QRec (New Zealand)
10. Isopropanol (99.7 %) – Qrec (New Zealand)
11. Methanol (99.8 %) – Qrec (New Zealand)
12. Methylene Blue (95.0 %) – UNILAB Reagent (Sydney, Australia)
13. Sodium Carbonate (99.0 %) – HmbG Chemicals (Hamburg, Germany)
14. Sodium Hydroxide (99.0 %) – SYSTERM (Selangor, Malaysia)
15. Sodium Nitrite (97.0 %) – AJAX Chemicals (Sydney, Australia)
16. Sunset Yellow FCF (90.0 %) – ALDRICH (Selangor, Malaysia)

2.1.2 Instruments

1. Analytical balance – SHIMADZU
2. Blender - Global
3. Fourier transform infrared (FTIR) spectrometer – Perkin Elmer 1600 model
4. Orbital shaker - WiseShake
5. pH meter – Denver Instrument
6. Rotary evaporation - Heidolph
7. Scanning electron microscope (SEM) with energy dispersive X-ray (EDX)
8. U.S.A. Standard Testing Sieve A.S.T.M. E-11 No.60 - Gilson Company Inc (250 mesh)
9. UV-Vis spectrophotometer - Hitachi U2000 with 1 cm length path quartz cell
10. Vacuum pump - Aspirator A-3 EYELA Tokyo
11. Water bath – WiseBath

2.1.3 Materials

1. *Uncaria gambir* cubes (Indonesia)

2.2 Characterization of *Uncaria gambir* Extract

2.2.1 Extraction of *Uncaria gambir*

Uncaria gambir cubes form Indonesia were ground with blender and then, were sieved through a 250 mesh sieve. The fatty materials of gambir were removed by using maseration extraction method where 60 g of gambir powders with 300 mL of hexane for 24 hours. Then, this defatted gambir was extracted again with maseration method where 1 g of defatted gambir will be extracted with 50 mL of different types of solvents (Table 2.1) at room temperature for 3 hours. Then, the solution was filtered. All extracts were concentrated by rotary evaporator. The equation for the calculation of fat percentage is given as:

$$\% \text{ Fat} = \frac{W_f \text{ (g)}}{W_g \text{ (g)}} \times 100 \tag{2.1}$$

where W_f is the fat content weight, W_g is the initial weight of gambir.

Table 2.1: Types of solvents and polarity index (John, 2003).

Solvent	Polarity Index
Isopropanol	3.9
Ethyl acetate	4.4
Acetone	5.1
50 % Methanol	6.6
Water	10.2

2.2.2 Fourier transform infrared (FTIR) spectroscopy

Raw gambir, standard catechin and gambir extracts were record with a Perkin Elmer System 2000 FTIR spectrometer. Pressed pellet was prepared by grinding the sample powder with the IR grade KBr in an agate mortar. The scanning wavenumber of infrared was at 4000–400 cm^{-1}.

2.2.3 Determination of total phenolic content

The total phenolic content was determined with the Folin-Ciocalteu method. In this method, 0.5 mL of sample and standard catechin were mixed with 5 mL of 10 % (v/v) FCR and 4 mL of 1 M sodium carbonate (Na_2CO_3) solution. Then, these solutions were diluted to 10 mL using distilled water. The mixtures were shaken thoroughly and then incubated for 2 hours in the dark at room temperature. The absorbance of all samples was measured at 765 nm using a UV–Vis spectrophotometer (JASCO V-530). The equation for total phenolic calculation is given as (Guno and Prashant, 2010):

$$\text{Phenolic content}, (\text{mg g}^{-1}) = \frac{C_f\ (\text{mg L}^{-1}) \times V\ (\text{L}) \times [V_i\ (\text{mL})/V\ (\text{mL})] \times \text{df}}{M\ (\text{g})} \qquad (2.2)$$

$$\%\ \text{Phenolic content} = \frac{C_f\ (\text{mg L}^{-1})}{C_i\ (\text{mg L}^{-1})} \times 100 \qquad (2.3)$$

where, C_i and C_f (mg L^{-1}) is the initial and final concentration of sample, V (L) is the volume of sample used for test, V_i (mL) is the volume of sample prepared, df is the dilution factor and M (g) is the mass of extract used.

2.2.4 Determination of flavonoid content

Distilled water, 4 mL was added into 10 mL volumetric flask and then it was added with 1 mL of diluted sample and standard catechin. At 0 min, 0.3 mL of 5 % $NaNO_2$ was added into flask. At 5 min, 0.3 mL of 10 % $AlCl_3$ was added and after 6 min, 2 mL of 1 M NaOH was added to the mixture. Immediately, 2.4 mL of distilled water was added to make up the mixture to be 10 mL. The absorbance was measured at 510 nm using a UV-Vis spectrophotometer (JASCO V-530). The equation for flavonoid content calculation is given as(Guno and Prashant, 2010):

$$\text{Flavonoid content}, (\text{mg g}^{-1}) = \frac{C_f\ (\text{mg L}^{-1}) \times \text{V (L)} \times [V_i\ (\text{mL})/\text{V (mL)}] \times \text{df}}{\text{M (g)}} \quad (2.4)$$

$$\%\ \text{Flavonoid content} = \frac{C_f\ (\text{mg L}^{-1})}{C_i\ (\text{mg L}^{-1})} \times 100 \quad (2.5)$$

where, C_i and C_f (mg L^{-1}) is the initial and final concentration of sample, V (L) is the volume of sample used for test, V_i (mL) is the volume of sample prepared, df is the dilution factor and M (g) is the mass of extract used.

2.2.5 Determination of Condensed Tannin

In 50 mL round bottom flask, 0.1 g of extract sample was dissolved with 10 mL of distilled water. Then, 1 mL of 10 M HCl and 2 mL of 37 % formaldehyde were added into the bottom flask. The mixture was refluxed for 30 minutes at 50 ºC. The suspension was filtered and washed with hot water to remove any residue. The precipitate was dried in an oven and weighted to obtain the yield (Tong, 2009). The equation to calculate the yields is given as:

$$\% \text{ Yield} = \frac{W_f \text{ (mg)}}{W_i \text{ (mg)}} \times 100 \qquad (2.6)$$

where, W_i (mg) is the initial weight of sample, and W_f (mg) is the final weight of sample.

2.2.6 Preparation of *Uncaria gambir* adsorbent

The modified gambir adsorbent (MGA) was prepared according to the Stiasny test method (Garro-Galvez et al., 1997). The gambir extract was collected and utilized for the preparation of adsorbent. The extract gambir was chemically treated with formaldehyde in an acidic medium under optimized conditions. For this, 0.1 g ethyl acetate gambir extract was added to 10 mL of distilled water, 2 mL of 37 % formaldehyde and 1 mL of 10 M HCl in a 50 mL conical flask with an attached condenser. After refluxed at 90 ºC for 1 hour the mixture was filtered out and washed with hot distilled water until the washings were of approximately pH 4. The modified gambir then was dried at 50 ^{o}C in an oven until a constant mass of the modified gambir adsorbent was obtained.