PREPARATION AND EVALUATION OF MANGROVE TANNINS-BASED ADSORBENT FOR THE REMOVAL OF HEAVY METAL IONS FROM AQUEOUS SOLUTION

by

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1/n	Adsorption intensity / heterogeneity factor
^{13}C CPMAS NMR	Carbon-13 Cross-Polarization and Magic-Angle Spinning Nuclear Magnetic Resonance
AAS	Atomic Absorption Spectrometry
b	Median association constant
BDDT	Brunauer, Deming, Deming and Teller
BET	Brunauer, Emmett and Teller
BJH	Barrett, Joyner and Halenda
Bt	Mathematical function of *F*
C_e	Equilibrium concentration in milligram per liter (mg/L)
C_f	Remaining metal ions concentration in milligram per liter (mg/L)
CHN	Carbon, Hydrogen and Nitrogen
C_o	Initial metal ions concentration in milligram per liter (mg/L)
DP_n	Degree of polymerization
D-R	Dubinin-Radushkevish
E	Mean energy of adsorption in kilojoule per mole (kJ/mol)
?	Polanyi constant
ESI-MS	Electrospray-Ionization-Mass Spectrometry
F	Function of adsorbate adsorbed at different time
FTIR	Fourier Transform-Infrared
HHDP	Hexahydroxydiphenic acid
HPLC	High Performance Liquid Chromatography

HPLC/DAD	High Performance Liquid Chromatography with Diode Array
HPLC/MS	High Performance Liquid Chromatography with Mass Spectrometry
K	Dubinin-Radushkevish constant in mole per kilojoule (mol/kJ)
K_1	First-order kinetic constant in one per minute (1/min)
K_2	Second-order kinetic constant in gram per milligram minute (g/mg min)
K_F	Adsorption capacity in milligram per gram (mg/g)
K_{id}	Intraparticle diffusion rate constant in milligram per gram square root minute (mg/g $min^{1/2}$)
K_L	Langmuir equilibrium constant in liter per milligram (L/mg)
K_{sp}	Solubility product constant
MALDI-TOF-MS	Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectroscopy
NMR	Nuclear Magnetic Resonance
Ph	Phenyl group
pH_{zpc}	pH zero point of charge
Q	Amount adsorbed at equilibrium in mole per gram (mol/g)
q_e	Amount adsorbed at equilibrium / adsorption capacity in milligram per gram (mg/g)
q_m	Total binding sites
Q_m	Maximum adsorption capacity in mole per gram (mol/g)
q_o	Amount adsorbed at initial in milligram per gram (mg/g)
q_t	Adsorption capacity at time *t* in milligram per gram (mg/g)
R	Gas constant in kilojoule per mole (kJ/mol)
R	Correlation coefficient

RMSE	Residue mean square error
S_{BET}	BET surface area
TBA	Tannins-based adsorbent
TBA(A)	Tannins-based adsorbent produced in acid-catalyzed condition
TBA(B)	Tannins-based adsorbent produced in base-catalyzed condition
TLC	Thin Layer Chromatography
TMS	Tetramethyl silane
UV-Vis	Ultraviolet visible
V_m	Monolayer adsorption capacity in milligram per gram (mg/g)

PENYEDIAAN DAN PENILAIAN PENJERAP BERASASKAN TANIN BAKAU UNTUK PENYINGKIRAN ION LOGAM BERAT DARIPADA LARUTAN AKUEUS

ABSTRAK

Struktur oligomer poliflavonoid tanin bakau yang diekstrak daripada kulit bakau *Rhizophora apiculata* dan prestasi penjerap berasaskan tanin (TBA) terhadap penjerapan ion logam berat daripada larutan akueus telah dikaji. Kulit bakau *Rhizophora apiculata* yang dikumpulkan merupakan hasil buangan daripada industri arang kayu di Kuala Sepetang, Perak, Malaysia. Sifat-sifat kimia tanin bakau yang diekstrak melalui kaedah pengekstrakan pepejal-cecair dengan menggunakan larutan akueus aseton/air (7:3, v/v) telah dianalisis. Kajian FTIR and NMR berkeadaan pepejal mengesahkan bahawa tanin bakau mengandungi prosianidin dalam nisbah yang tinggi berbanding dengan prodelfinidin dengan rangkaian dominan antara flavonoid adalah C4-C8 dan konfigurasi *cis*- di C2 dan C3 membentuk kumpulan oligomer yang besar. Analisis MALDI-TOF bagi tanin bakau *Rhizophora apiculata* menunjukkan bahawa tanin mengandungi oligomer prosianidin yang terdiri daripada katecin/epikatecin, epigallokatecin dan epikatecin gallat wujud dalam nisbah yang besar. Oligomer tanin mencapai sehingga nonamer dengan unit ulangannya adalah 528 – 529 Da merupakan dimer katecin gallat yang telah kehilangan kedua-dua residu asid gallik dan kumpulan hidroksil merupakan spesies dominan. Seterusnya wujud oligomer tanin untuk kedua-dua jenis yang dihubung secara kovalen. TBA yang dihasilkan daripada prarawatan tanin bakau dengan formaldehid dalam keadaan bermangkin asid, TBA(A) dan bes, TBA(B) telah dicirikan dengan analisis FTIR,

NMR berkeadaan pepejal dan unsur CHN menunjukkan pembentukan rangkaian metilin selepas prarawatan. TBA(A) dan TBA(B) merupakan penjerap berdominankan liang meso dan masing-masing mempunyai pH_{zpc} 3.45 dan 4.09. Penjerapan ion logam kuprum (II), plumbum (II), kromium (III) and (VI) pada TBA(A) dan/atau TBA(B) telah dinilai. Kesan daripada beberapa parameter seperti pH awalan, dos penjerap, kepekatan awalan ion logam dan masa persentuhan telah dikaji. Untuk kes-kes penjerapan ion logam kuprum (II), kromium (III) dan plumbum (II), pH penjerapan optimum adalah melebihi pH_{zpc} TBA(A) dan/atau TBA(B). Ion kromium (VI) pula menunjukkan pH penjerapan optimum di bawah pH_{zpc} TBA(B). Data penjerapan pada keseimbangan telah dipadankan dengan model-model isoterma seperti Langmuir, Freundlich, Sips dan Dubinin-Radushkevich. Penjerapan heterogenus ion-ion logam berat yang dikaji menunjukkan kapasiti penjerapan lapisan tunggal mengikuti turutan ion logam plumbum (II) > kromium (VI) > kromium (III) > kuprum (II). Purata tenaga penjerapan (*E*) untuk ion logam yang dikaji adalah di antara 8 dan 16 kJ/mol iaitu dalam julat tenaga untuk tindak balas penukaran ion kecuali untuk ion kuprum (II). Penjerapan fizikal juga terlibat dalam penyingkiran ion kuprum (II). Kajian kinetik menunjukkan penjerapan ion logam yang diselidik mengikuti tindak balas tertib kedua dengan kehadiran pembauran filem dan antara partikel dengan proses penjerapan adalah dikawal oleh pembauran filem.

PREPARATION AND EVALUATION OF MANGROVE TANNINS-BASED ADSORBENT FOR THE REMOVAL OF HEAVY METAL IONS FROM AQUEOUS SOLUTION

ABSTRACT

The polyflavonoid oligomeric structures of mangrove tannins extracted from the barks of *Rhizophora apiculata* and performance of tannins-based adsorbent (TBA) on the adsorption of heavy metal ions from the aqueous solutions were explored. The barks of *Rhizophora apiculata* mangrove were collected as the waste product from the charcoal industry in Kuala Sepetang, Perak, Malaysia. Mangrove tannins extracted with acetone/water (7:3, v/v) by using the solid-liquid extraction method were subjected to various analyses to characterize their chemical properties. FTIR and solid-state NMR study confirmed that mangrove tannins have high proportions of procyanidins to prodelphinidins with the predominant interflavonoid linkages of C4-C8, and flavonoid units with *cis*-configuration at C2 and C3 formed the bulk of the oligomers. The MALDI-TOF-MS analysis showed that the *Rhizophora apiculata* mangrove tannins consisted of procyanidin oligomers formed by catechin/epicatechin, epigallocatechin and epicatechin gallate monomers are present in great proportions. Oligomers in tannins up to nonamers, in which the repeating unit at 528 – 529 Da is a catechin gallate dimer that has lost both the gallic acid residues and a hydroxyl group are predominant species. Futhermore, oligomers of the two types covalently linked to each other also occur. TBA produced from the pretreatment of mangrove tannins with formaldehyde in acid-catalyzed condition, TBA(A) and base-catalyzed condition, TBA(B) were characterized by FTIR, solid-state NMR and CHN elemental analysis showed the formation of methylene bridges after the pretreatment. TBA(A) and TBA(B) which were predominantly mesoporous adsorbents have pH_{zpc} of 3.45 and 4.09, respectively. The adsorption of copper (II), lead (II), chromium (III) and (VI) metal ions on TBA(A) and/or TBA(B) in batch adsorption experiments was evaluated. The effect of several parameters like initial pH, adsorbent dosages, initial metal ions concentrations and contact time were investigated. In the case of the adsorption of copper (II), chromium (III) and lead (II) metal ions, the optimum adsorption pH were above the pH_{zpc} of TBA(A) and/or TBA(B). Chromium (VI) ions, however, showed optimum adsorption pH below the pH_{zpc} of TBA(B). The adsorption equilibrium data was fitted with isotherm models such as Langmuir, Freundlich, Sips and Dubinin-Radushkevich isotherm models. Heterogeneous adsorption of the studied heavy metal ions have the monolayer adsorption capacity that followed the order of lead (II) > chromium (VI) > chromium (III) > copper (II) metal ions. The mean adsorption energies (*E*) were found to be between 8 and 16 kJ/mol for the studied heavy metal ions which are in the energy range of ion exchange reaction except for copper (II) ions. Physisorption was involved in the removal of copper (II) ions as well. Kinetic study showed that the adsorption of the studied heavy metal ions followed pseudo second-order reactions with the presence of film and intraparticle diffusion in which the adsorption process was governed by film diffusion.

CHAPTER ONE

INTRODUCTION

1.1 Tannins

The term “tannins” is no longer strange in chemistry field and it comes from the ancient Celtic word for oak which was a typical source of tannins for leather making (Bisanda *et al*., 2003; Hagerman, 2002). According to Khanbabaee and van Ree (2001), the name “tannin” is derived from the French word – tanin which means a tanning substance and is used for a range of natural polyphenols. The ancient society had been using tannins to convert animal skin to form leather as tannins are able to interact with and precipitate proteins, including the protein found in animal skin (Hagerman, 2002; Khanbabaee and van Ree, 2001).

Tannins are secondary metabolites widely found in plant kingdom and produced via condensation of simple phenolics (Chavan *et al*., 2001). Although tannins themselves are secondary phenolic metabolites, their chemical reactivities and biological activities have distinguished them from other plant secondary phenolics (Hagerman, 2002). Many researchers have tried to define tannins based on their structures, chemical reactivities and biological activities. The complexity of tannins, however, has hindered their efforts to provide an appropriate definition for tannins. Bates-smith and Swain (1962) defined tannins as water soluble phenolics with molecular weights between 300 and 3000 Daltons (Da), exhibit usual phenolic reactions and showing the ability to precipitate alkaloids, gelatins and other proteins. This definition, however, does not include all tannins since tannins with higher molecular weight of up to 20000 Da have been isolated. Griffith (1991) described tannins as "macromolecular phenolic substances" and divided them into two main groups namely hydrolysable tannins and condensed tannins. His definition of tannins only covered tannins with high molecular weight and ignores the low molecular and monomeric tannins with a molar mass below 1000 Da (Khanbabaee and van Ree, 2001). Haslam (1989) in an attempt to emphasize the multiplicity of phenolic groups characteristic of tannins has substituted the term "polyphenol" for "tannin". He noted that tannins with molecular weight up to 20000 Da have been reported and tannins complex not only with proteins and alkaloids but with certain polysaccharides as well (Hagerman, 2002).

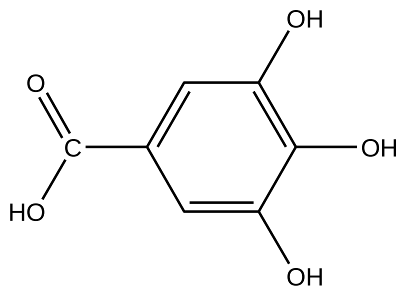
Recent work by Khanbabaee and van Ree (2001) based on the molecular structures of known tannins, their origin and role in plant life have defined tannins as polyphenolic secondary metabolites of higher plants, and are either galloyl esters and their derivatives; in which galloyl moieties or their derivatives are attached to a variety of polyol-, catechin- and triterpenoid cores (gallotannins, ellagitannins and complex tannins), or they are oligomeric and polymeric anthocyanidins that can possess different interflavanyl coupling and substitution patterns (condensed tannins).

1.1.1 Classification of tannins

The complexities of tannins do not constitute a unified group, but they exhibit a variety of molecular structures. The tannins containing poly-hydroxylphenyl groups (Nakano *et al*., 2001) can exist in different forms of oligomers and polymers. Based on their chemical structures and behavior, tannins generally can be categorized into two large groups, hydrolysable tannins and condensed tannins (Chavan *et al*., 2001; Nakamoto *et al*., 2003; Okuda *et al*., 1989).

1.1.1.1 Hydrolysable tannins

This class of tannins has polyol, usually D-glucose as the centre core and it is the starting point for many complex tannins structures. Gallic acid (**1**) and hexahydroxydiphenic acid (HHDP) (**2**) groups represent the polyphenolic parts in the molecules of hydrolysable tannins. The hydroxyl functions of the centre core polyol maybe partly or fully substituted through esterification with galloyl units to yield gallotannins like pentagalloyl glucose (**3**). Those having the HHDP groups have been named as ellagitannins like casuarictin (**4**) (Okuda *et al*., 1989). In gallotannins, the centre core polyol is surrounded by several gallic acid units. Further gallic acid units can be attached through a depside bond as shown in Fig. 1.1 (Mueller-Harvey, 2001). Ellagitannins are found from the oxidative coupling of galloyl groups on gallotannins and the simple ellagitannins are esters of HHDP. Hydrolysable tannins are susceptible to hydrolysis by acids, bases or esterase. Hydrolysis of gallotannins with strong acids will yield gallic acid and the core polyol. Meanwhile, hydrolysis of ellagitannins will liberate HHDP which spontaneously lactonized to ellagic acid (**5**) in aqueous solution (Hagerman, 2002).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HO
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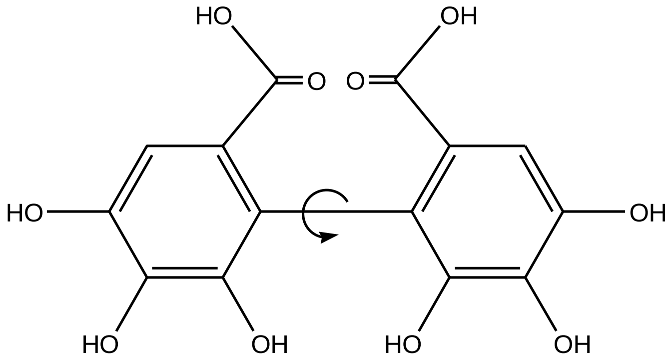
1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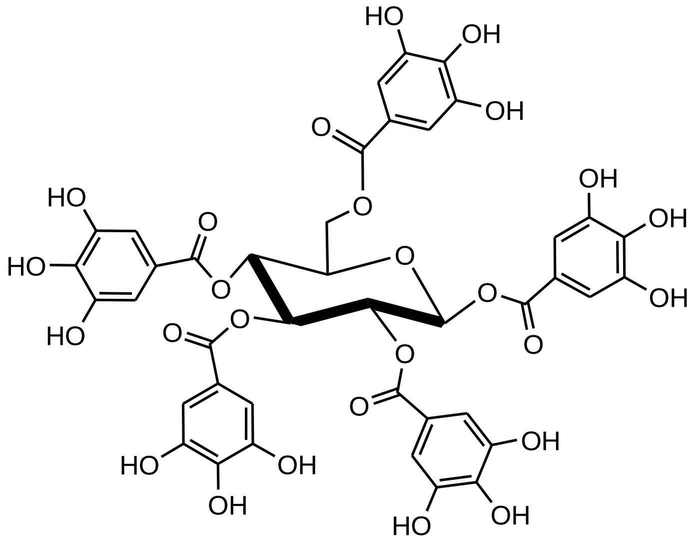
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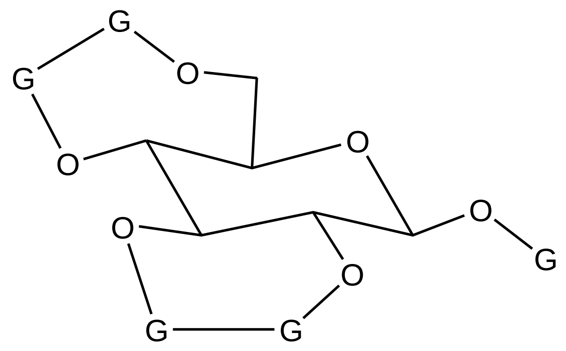
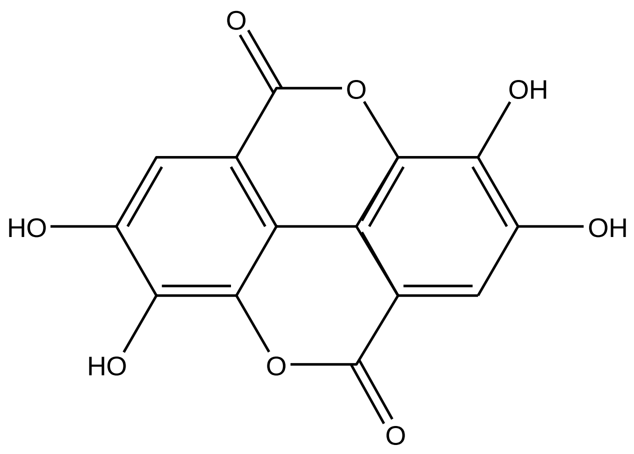


G = galloyl

4



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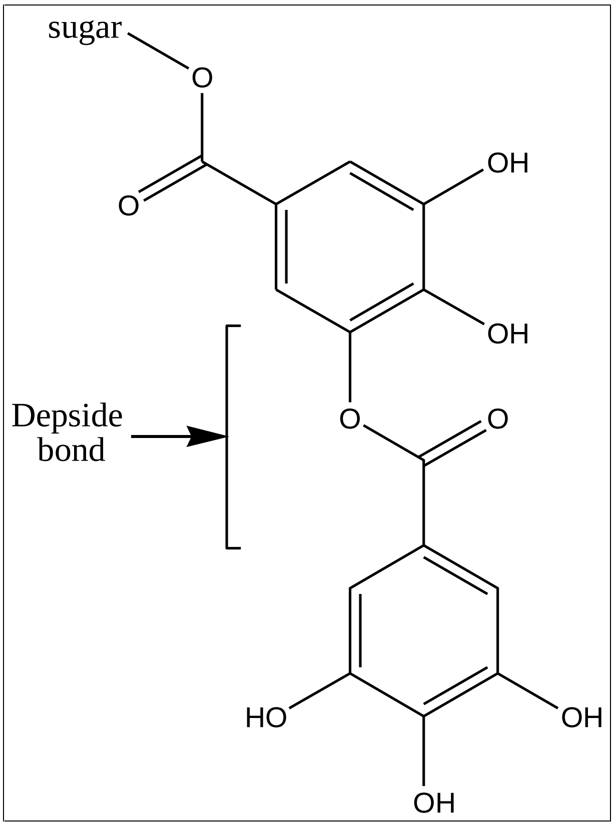
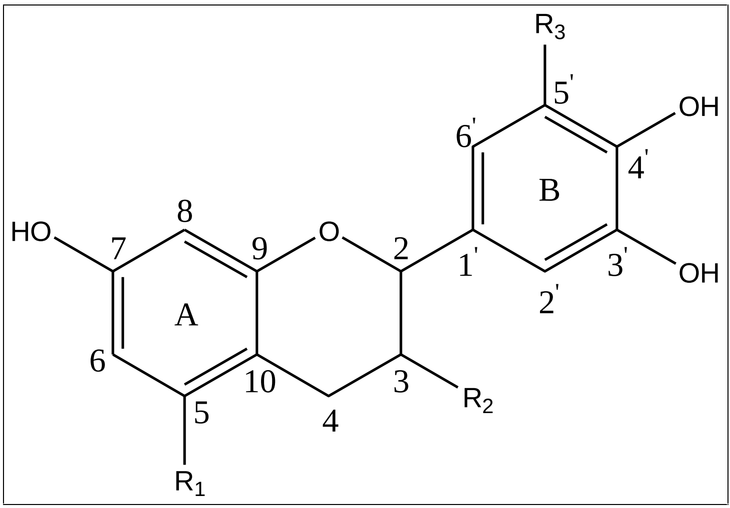


Fig. 1.1 Depside bond which is formed between the phenolic group of the upper and acid group of the lower gallic acid units (Mueller-Harvey, 2001).

1.1.1.2 Condensed tannins

Condensed tannins also known as proanthocyanidins are polymeric flavonoids (Hagerman, 2002). They comprise a group of polyhydroxy flavan-3-ol oligomers and polymers linked by carbon-carbon bonds between the flavonoid subunits (Schofield *et al*., 2001). The structure of flavonoid subunit, the standard letters to identify the rings and the numbering system are as shown in Fig. 1.2 (Hagerman, 2002; Pizzi, 1994). Basically, flavonoid units in condensed tannins present phloroglucinol (R_1 = OH) or resorcinol (R_1 = H) A-rings and catechol (R_3 = H) or pyrogallol (R_3 = OH) B-rings. Polymeric condensed tannins composing of fisetinidin (resorcinol A-ring, catechol B-ring) (**6)** and robinetinidin (resorcinol A-ring, pyrogallol B-ring) (**7**) are called profisetinidin and prorobinetinidin, respectively. When they are composed of catechin (phloroglucinol A-ring, catechol B-ring) (**8**) and gallocatechin (phloroglucinol A-ring, pyrogallol B-ring) (**9**), the polymers are called procyanidin and prodelphinidin, respectively (Pizzi, 1994). The hydroxyl group at position R_2 sometime esterified with gallic acid to yield e.g. epigallocatechin gallate (R_1 = R_3 = OH, R_2 = O-galloyl) (Schofield *et al*., 2001).



(**6**) R_1 = H; R_2 = OH; R_3 = H
(**7**) R_1 = H; R_2 = OH; R_3 = OH
(**8**) R_1 = OH; R_2 = OH; R_3 = H
(**9**) R_1 = OH; R_2 = OH; R_3 = OH

Fig. 1.2 Structure of flavonoid subunit, the standard letters to identify the rings, and the numbering system.

Flavonoid units in condensed tannins are linked together through the C4 – C6 or C4 – C8 interflavonoid linkages. The former linkage is predominating in profisetinidin and prorobinetinidin. Meanwhile, C4 – C8 interflavonoid linkages are predominant in procyanidin and prodelphinidin (Pizzi, 1994). A typical structure model of condensed tannins is shown in Fig. 1.3. In general, condensed tannins possess rigid carbon-carbon interflavonoid bonds that cannot be broken easily by hydrolysis (Bisanda *et al*., 2003).

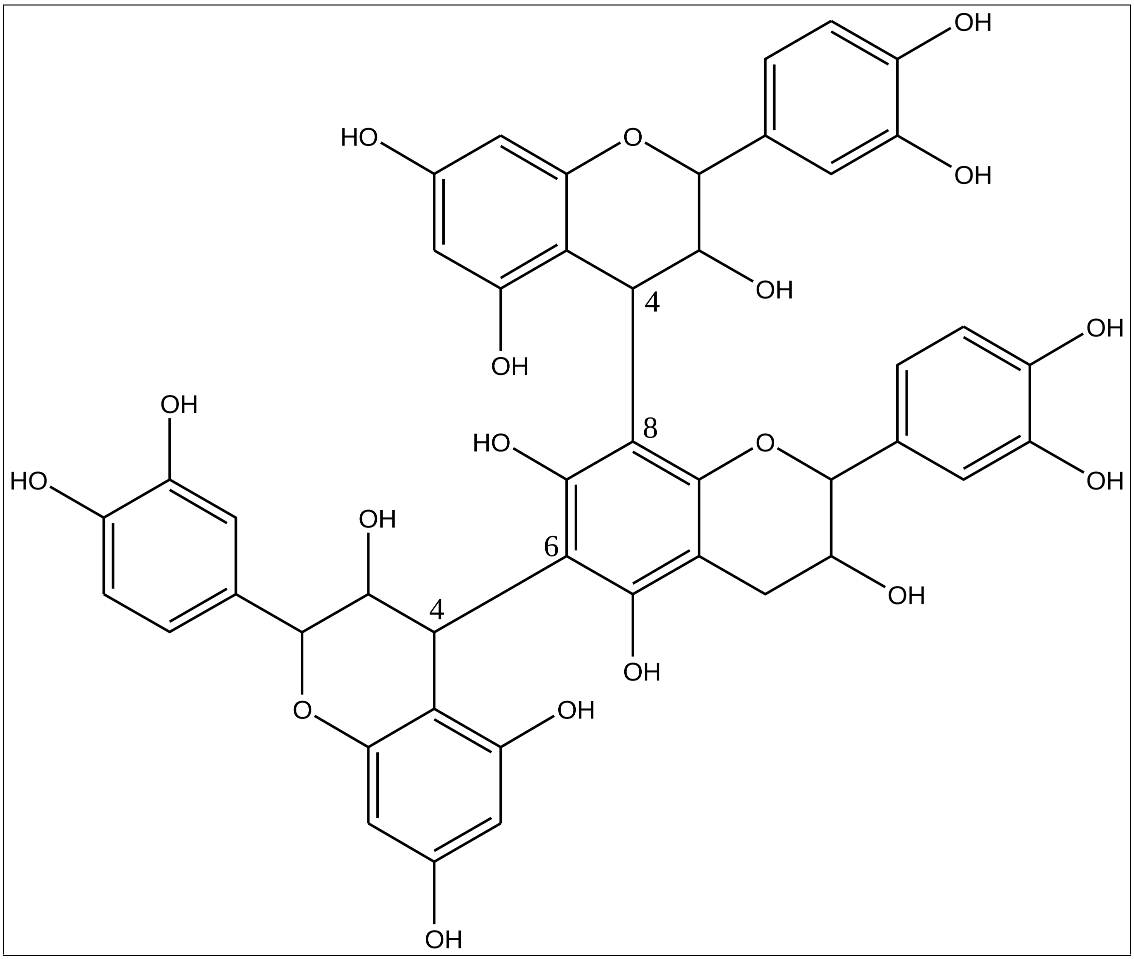


Fig. 1.3 Typical structure of condensed tannins with C4 – C8 and C4 – C6 interflavonoid linkages.

1.2 Sources of tannins

Tannins are widely spread in plant kingdom. They are synthesized by a wide variety of plants and trees such as *Acacia* sp. (wattle), *Eucalyptus* sp., *Mirtus* sp. (myrtle), *Acer* sp. (maple), *Betula* sp. (birch), *Salix* Caprea (willow), *Pinus* sp. (pine) and many more (Bisanda *et al*., 2003). Tannins can be extracted from different parts of plants and are located mainly in the vacuoles or surface waxes of the plants where they do not interfere with the plant metabolism. Location of tannins in various plant tissues are (Tannins, 2001):

- Bud tissues – most common in outer part of the bud, probably as protection against freezing.
- Leaf tissues – most common in the upper epidermis. However, in evergreen plants, tannins are evenly distributed in all leaf tissues. They serve to reduce palatability and, thus, protect against predators.
- Root tissues – most common in the hypodermis (just below the supervised epidermis). They probably act as a chemical barrier to penetration and colonization of roots by plant pathogens.
- Seed tissues – located mainly in a layer between the outer integument and aleurone layer. They have been associated with the maintenance of plant doemancy, and have allelopathic and bactericidal properties.
- Stem tissues – often found in the active growth area of trees, such as the secondary phloem and xylem and the layer between epidermis and cortex. Tannins may have a role in the growth regulation of these tissues. They are

also found in the heartwood of conifers and may be contributed to the natural durability of wood by inhibiting microbial activity.

Researchers have extracted tannins from bark of *Pinus radiata* (Palma *et al*., 2005); leaf of Sumac (*Rhus coriaria* L.) (Zalacain *et al*., 2003); seeds of *Pigeon pea* (Ferreira *et al*., 2004); flowers and leaves of *Crataegus* (Svedström *et al*., 2002); membrane of pecan (*Carya illinoensis*) nut, bark of mimosa (*Acacia mearnsii*) and wood of quebracho (*Schinopsis balansae*) (Pasch *et al*., 2001), etc. In Malaysia, tannins have been extracted from *Phyllanthus niruri* or known as "dukung anak" (Markom *et al*., 2006) and mangrove barks of *Rhizophora apiculata* (Oo *et al*., 2008; Rahim *et al*., 2006).

1.3 Mangrove in Malaysia

The mangrove trees have served as one of the sources of tannins in Malaysia. Mangrove forests are well developed along the coastline of Malaysia, especially along the sheltered estuaries and deltas at west coast of Peninsular Malaysia that facing the Straits of Malacca. Malaysia has a vast area of mangrove forest and in year 2003, mangrove forests recorded a total area of 566,866 ha., 60 % are in Sabah, 22.3 % in Sarawak and 17.6 % in Peninsular Malaysia. Islands near to the coastlines with well-grown mangrove forests are the six islands that made up the archipelago of Pulau Klang and Pulau Kukup in Johor. The reserved mangrove forests in Malaysia are under the management of Forestry Department to ensure the controlled production of wood. Malaysia today is practicing clear-filling logging system in

Fig. 1.4 Matang Mangrove Forest Reserve in Perak. Matang Mangrove Forest (a), *Rhizophora apiculata* mangrove tree (b) and (c).

which replanting of mangrove trees is done by following the cycle of 20 – 30 years. In Peninsular Malaysia, the Matang Mangrove Forest Reserve in Perak (Fig. 1.4a) covered the area of 40,151 ha. The systematic Matang Mangrove Forest management has been acknowledged by the international body as the most well managed system in the world and the forest is serving as the source for charcoal, firewood and construction materials like wood pillars (Bakau, 2005). Matang Mangrove Forest Reserve is about 51 km of coastline and 13 km wide, it stretched from Kuala Gula in the north to Bagan Panchor in the south (Rahim, 2005). A variety of vegetation can be found in Matang Mangrove Forest Reserve, among them are *Rhizophoraceace*. Two main types of *Rhizophoraceace* species are *Rhizophora apiculata* (bakau minyak) (Fig. 1.4b, c) and *Rhizophora mucronata* (bakau kurap).

1.4 Tannins from *Rhizophora apiculata* mangrove barks

The barks of *Rhizophora apiculata* mangrove (Fig. 1.5) are the waste product from the charcoal industry. The Kuala Sepetang Charcoal Village that is located by the Matang Mangrove Forest Reserve has started to exploit mangrove timber for charcoal production since 1930 with the introduction of the charcoal kiln (Chan, 1985). According to one of the owners of the charcoal factories, between the two *Rhizophoraceace* species in Matang Mangrove Forest Reserve, only *Rhizophora apiculat*a is allowed to be harvested for charcoal production when they reached the age of 30 years. In the charcoal making process, the mangrove logs are debarked prior to heating in the kilns as the barks contain high content of water and will interfere with the heating process. The barks of *Rhizophora apiculata* mangrove then

Fig. 1.5 Barks of *Rhizophora apiculata* mangrove as the waste product from the charcoal industry.

as the waste product will be burned off. Studies have showed that high content of raw tannins up to 34.68 %(w/w) could be extracted from the barks of *Rhizophora apiculata* mangrove based on the 3 days of solid-liquid extraction with 70 %(v/v) aqueous acetone (Yeong, 2003). Barks *of Rhizophora apiculata* mangrove are a good source of tannins as they are low in cost and abundantly available from the environment. The extraction of tannins from the mangrove barks involved simple and inexpensive methods, thus they have a great potential of commercial value.

1.4.1 Study on *Rhizophora apiculata* mangrove tannins

Isolation, identification and characterization of tannins from different sources have been conducted by many researchers. Foo (1981) has studied the B-ring hydroxylation pattern and the configuration of the flavonoid units of condensed tannins by using IR spectroscopy. Svedström *et al*. (2002) has performed electrospray-ionization-mass spectrometry (ESI-MS), TLC and HPLC analysis on oligomeric procyanidins isolated from the leaves and flowers of hawthorn (*Crataegus laevigata*); HPLC/DAD and HPLC/MS qualitative analysis of condensed and hydrolysable tannins of commercial extract of pine barks *(P. maritime* L.) were conducted by Romani *et al*. (2006); Detection and characterization of proanthocyanidins from *Quercus petraea* and *Q. robur* heartwood by using HPLC and ^{13}C NMR were conducted by Vivas *et al*. (2006); Characterization of high-tannin fractions from humus by ^{13}C NMR with cross-polarization and magic-angle spinning (CPMAS) was done by Lorenz and Preston (2002); Pasch *et al*. (2001) have elucidated the polymeric structure of quebracho and mimosa tannins by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) mass spectroscopy (MS).

Tannins have been extracted from *Rhizophora apiculata* mangrove barks from Matang Mangrove Forest Reserve for various application purposes. However, not many efforts were done to study the chemistry of *Rhizophora apiculata* mangrove tannins. A study on the mangrove barks from Tarakan, East Kalimantan reported a total amount of 14.9 %(w/w) of tannins were extracted from the *Rhizophora apiculata* mangrove barks with the percentage of catechin as high as 14.4 %(w/w) (Achmadi and Choong, 1992). Recent study by using reversed-phase HPLC showed that tannins extracted from *Rhizophora apiculata* mangrove barks from Matang Mangrove Forest Reserve constitute mainly of four flavonoid monomers namely catechin, epicatechin, epigallocatechin and epicatechin gallate, which made the *Rhizophora apiculata* mangrove tannins a condensed type (Rahim *et al*., 2006). This study has managed to isolate and identify the monomeric flavonoid structures of *Rhizophora apiculata* mangrove tannins. However, no work has been carried out to determine the molecular weight and degree of polymerization of *Rhizophora apiculata* condensed tannins. Studies had showed that *Rhizophora apiculata* mangrove tannins have great potential as alternative steel corrosion inhibitors (Rahim *et al*., 2006) and as adsorbent for heavy metal ions in aqueous solution (Oo and Jain, 2007). Thus it is important to investigate the polyflavonoid tannin oligomers by using an appropriate analytical method like Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) mass spectroscopy (MS). Meanwhile, the linkages between the flavonoid units can be studied by NMR technique.

Since its introduction by Karas *et al.* (1987), Matrix-Assisted Laser Desorption/Ionization (MALDI) mass spectroscopy has greatly expanded the use of mass spectroscopy towards the analysis of large molecules. MALDI mass spectroscopy itself has revealed as a powerful method for the characterization of both synthetic and natural polymers. Fragmentation of the analyte molecules upon laser irradiation can be substantially reduced by embedding them in a light adsorbing matrix. As a result, intact molecules are desorbed and ionized along with the matrix and can be analyzed in a mass spectrometer. This technique is mostly coupled with time-of-flight (TOF) mass analysers. This is so as TOF-MS present the advantage of being capable to provide a complete mass spectrum per event, for its virtually unlimited mass range, for the small amount of analyte needed and the relatively low cost instrument (Pasch *et al*., 2001). Other advantages of MALDI-TOF MS are only one molecular ion is formed from each parent molecule, high sensitivity across a broad range of masses allows detection of oligomeric series of compounds, the ability to detect compounds with high molecular weight, and interpretation of isotopes patterns allows the detection of oligomers with small differences in mass. MALDI-TOF MS is ideally suited for characterizing polydispersed oligomers and considered as the mass spectrometric method of choice for analysis of tannins which exhibit large structural heterogeneity (Reed *et al*., 2005). Recently, the polymeric structures of tannins from different plant species were studied by using MALDI-TOF MS (Ishida *et al*., 2005; Xiang *et al*., 2006).

Mass spectrometry method like MALDI-TOF MS can provide information on the components and molecular weight of the polymeric tannins. However, the linkages between the components are best studied by using the NMR technique (Mueller-Harvey, 2001) such as solid-state NMR. Solid-state NMR is not only widely used for characterization of crystalline powder but also amorphous samples and polymers, including various plant materials (Wawer *et al*., 2006). Characterization of tannins structures by using ^{13}C NMR has been done by many researchers like Lorenz and Preston (2002), Newman and Porter (1992) and Vivas *et al*. (2006).

1.5 Utilization of tannins

The ancient society had started to use tannins on the traditional manufacture of leather from animal skins by tanning them with extracts of certain woody plants (Achmadi and Choong, 1992; Bisanda *et al*., 2003; Harborne, 1984; Khanbabaee and van Ree, 2001; Whiting, 2001). The ability of tannins to cross-link with the protein enables them to transfer raw animal skins into leather. Real tanning is described as the cross linking of the skin's collagen chains, while false tanning involves the filling of hollow spaces between the skin's collagen chains (Khanbabaee and van Ree, 2001). The blue-black iron tannate complex was used by ancient Egyptians as a hair dye, and for many centuries, this complex was the main source of the writing inks (Slabbert, 1992).

The petroleum crisis of the early seventies have initiated the interest in seeking a return to plant-based panel adhesives as economical alternatives to petrochemical adhesives such as phenol and urea-formaldehyde (Manas, 1982; Wheatley, 1992). Tannins are phenolic compounds that can be used as adhesive materials. Tannins-formaldehyde adhesives are obtained by hardening of polymeric flavonoids of natural origin, of condensed tannins by polycondensation with formaldehyde (Pizzi, 1994). Since the introduction of tannins as a substitute of phenol in the production of adhesive resins, many efforts have been done to improve the tannins-formaldehyde resins. The development from researches has led to the commercial production of resins of low viscosity and with reactivity at least equal to that of urea-formaldehyde resins. Tannins-formaldehyde adhesive resins have been applied in the production of plywood and particleboard and used in glulam, finger jointing, and boat construction (Pizzi, 1983a).

Other uses of tannins are in the preservative treatment of fishing nets (Achmadi and Choong, 1992; Bakau, 2005); in the dyestuff industry as caustics for cationic dyes (tannin dyes), and also in the production of inks (iron gallate ink); in food industry to clarify wine, beer, and fruit juices. Other industries used tannins as textile dye, as antioxidant in the fruit juices, beer, and wine industries, and as coagulant in rubber production. In medicinal uses tannins containing plants extracts are used as astringents, against diarrhea, as diuretics, against stomach and duodenal tumors, and as anti-inflammatory, antiseptic, and haemostatic pharmaceuticals (Khanbabaee and van Ree, 2001). Condensed tannins also showed some physiological effects, such as antioxidant, anti-allergy, anti-hypertensive and antimicrobial activities in biological systems (Romani *et al*., 2006). The antioxidant property of tannins also enables them to be used as alternative steel corrosion inhibitors (Loo *et al*., 2007; Rahim *et al*., 2006).

There has lately been a growing interest in biosorbents, especially tannins-based adsorbent for removing a low concentration of heavy metal ions from aqueous solutions (Kim and Nakano, 2005; Liao *et al*., 2004; Ogata and Nakano, 2005). The development of biosorbents to remove heavy metal ions from the aqueous solution is important to replace the conventional methods like precipitation, membrane filtration, electrolyte or liquid extraction, electrodialysis and reverse osmosis (Gode and Pehlivan, 2005; Palma *et al*., 2003; Vázquez *et al*., 2002; Yamaguchi *et al*., 1992). These conventional methods are only successfully applied to solutions with high concentration of heavy metal ions. At low concentration, these methods are much less efficient and considerably expensive (Vázquez *et al*., 2002). Thus, a more economical, easily available and effective adsorbent to remove heavy metal ions at low concentration by mean of sorption technique is needed.

1.6 Tannins-based adsorbent for heavy metal ions

The presence of multi adjacent phenolic hydroxyls groups enable tannins to remove heavy metal ions from aqueous solution effectively (Liao *et al*., 2004; Nakajima, 2002; Santana *et al*., 2002). The ability of tannins to complex with metal ions is due to the ortho-hydroxyls present in their B-rings and it was found that ion exchange was the main adsorption mechanism as metal cations displace the adjacent phenolic hydroxyl groups forming a chelate (Vázquez *et al*., 1994; Zhan and Zhao, 2003). Tannins in nature are able to react with heavy metal ions in aqueous solutions, however, their solubility in water restrict their function as metal ions adsorbent in aqueous solutions (Liao *et al*., 2004). In order to overcome this disadvantage, tannins need to be immobilized by chemical modification. This is done through the reaction of tannins with formaldehyde. The main role of formaldehyde in the modification reaction is to immobilize the phenolic polymers in the tannins so that the water is not dyed, and the tannins ability to adsorb metals is improved by chemical modification as well (Palma *et al*., 2003).

Tannins as phenolic compounds are expected to react with formaldehyde as phenols under acid or base catalyzed conditions (Pizzi, 1983b). The reaction of phenol and tannins with formaldehyde are shown in Fig. 1.6 and 1.7, respectively. Tannins are reactive with formaldehyde due to the strong nucleophilic of their A-rings (Zhan and Zhao, 2003). Resorcinoric and phloroglucinoric nuclei of tannins A-rings ensure high reactivity of tannins towards formaldehyde; under parity conditions, which are between 10 to 50 times faster than the reaction of phenol with formaldehyde (Pizzi, 1994). The produced tannins-based adsorbent is insoluble in water, acidic and basic conditions (Shirato *et al*., 1994). The tannins-based adsorbent is a compound that consists of only carbon, hydrogen and oxygen, thus the volume after drying and incineration is reduced. Generally, the residue after the incineration is the oxide of the metal adsorbed (Matsumura and Usuda, 1998).

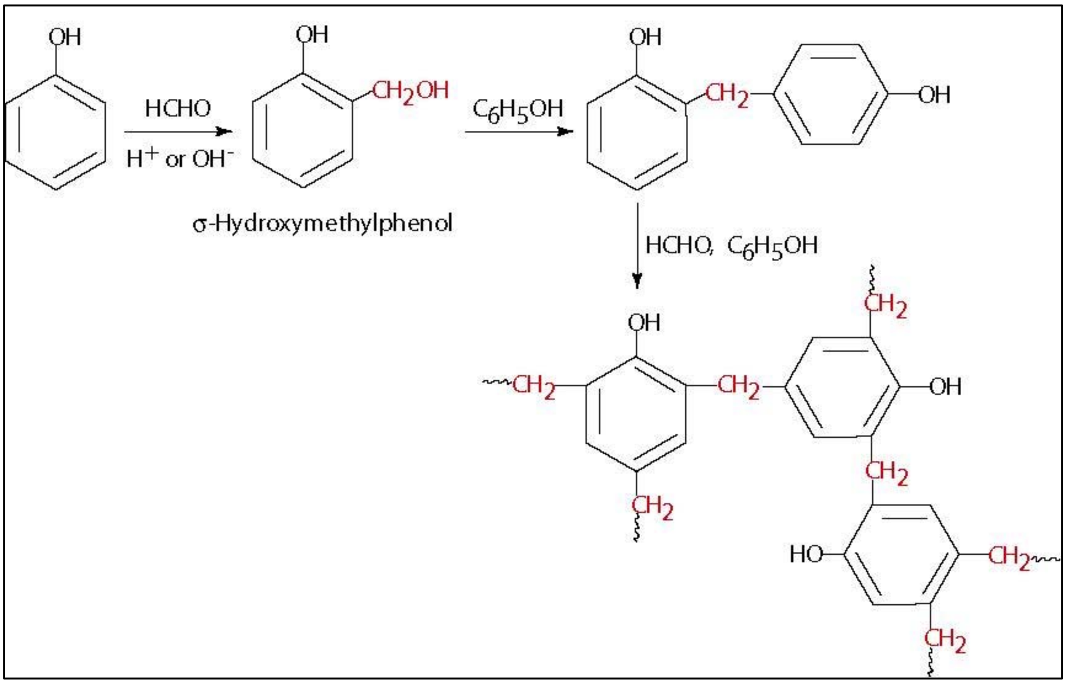


Fig. 1.6 Typical reaction between phenol and formaldehyde under acid- or base-catalyzed condition (Pizzi, 1983b).

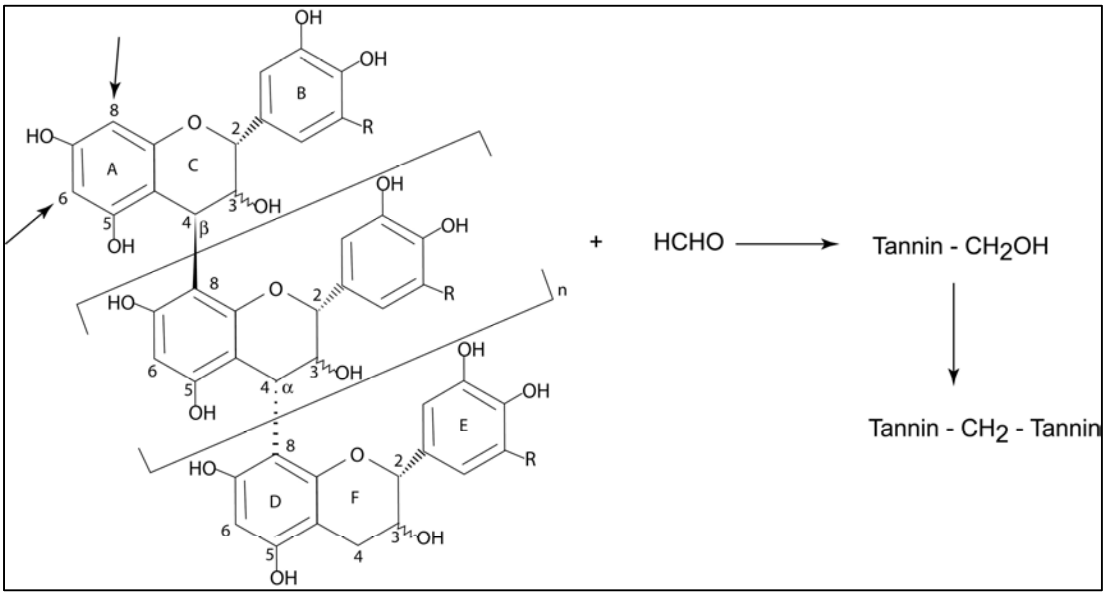


Fig. 1.7 Reaction of tannins with formaldehyde (arrow pointed are the reactive sites of tannins with formaldehyde) (Pizzi, 1983b).

1.7 Adsorption

The term "adsorption" was introduced by Kayser in 1881 to connote the condensation of gases on free surfaces, in contra-distinction to gaseous absorption where the molecules of gas penetrate into the mass of the absorbing solid (Gregg and Sing, 1967a). Atkin and Paula (2002) have defined adsorption as the attachment of particles to a surface. The substance that adsorbs is the adsorbate and the underlying material is the adsorbent or substrate. According to Oscik (1982a), 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. Adsorption processes are usually classified based on the kind of phases constituting the interphase, such as liquid/gas, solid/gas, solid/liquid and liquid/liquid, and according to the type of forces acting at this surface like physical adsorption (physisorption) and chemical adsorption (chemisorption).

Physisorption involves intermolecular forces such as van der waals forces, hydrogen bonds and etc. Meanwhile, chemisorption involves valency forces as a result of sharing of electrons by the solid (adsorbent) and the adsorbed substances (adsorbate) (Oscik, 1982b). In chemisorption, the adsorbates adsorbed to the surface by forming a chemical (usually covalent) bond, and tend to find sites that maximize their coordination number with the adsorbent (Atkins and Paula, 2002). Both physisorption and chemisorption can be distinguished by (Oscik, 1982b):

1. Heat of adsorption – small in the case of physisorption, large (of the same order as the heat of the relevant chemical reaction) in the case of chemisorption.
2. Reversibility – the adsorbed substance can be relatively easily removed from the surface when physisorption is involved; the removal of chemically adsorbed layer is very difficult and requires drastic measures.
3. Thickness of adsorbed layer – in the case of physisorption, under suitable conditions, adsorbed layers are formed having thicknesses of several diameters of the adsorbate molecule; in chemisorption only monolayers are formed.

1.7.1 Applications of adsorption

Adsorption process has been widely applied in many fields of modern industry, techniques and it even play an important role in our daily life. The fundamental practices applications of adsorption and related areas are as followings (Dabrowski, 2001):

- Separation and purification of liquid and gas mixtures, bulk chemicals, isomers and air;
- Drying gases and liquid before loading them into industrial systems;
- Removal of impurities from liquid and gas media;
- Recovery of chemicals from industrial and vent gases; and
- Water purification.

In petroleum industry, adsorption processes are important in purification of various petroleum products like fuel, oil, extraction benzenes, etc. Adsorption of proteins has been used in food production for a long time. New applications of the adsorbed proteins boost successful development of biotechnology, pharmacology and medicine, determining the usefulness of novel drugs and the control of drug administrations. In laboratory practice, adsorption technique is used in chromatography for analysis and separation mixtures with simultaneous evolution of high purity components. Adsorption also plays a significant role in neutralization of waste gases and sewages and at the same time capturing the valuable components found in the waste. In water treatment, adsorption plays an important role in removal of trace heavy metal ions such as Cd, Cr, Hg, Cu, Fe, V, Zn and Ni. The adsorption-related separation methods like ion-exchange are an important part of the effective removal of heavy metal ions and radioactive wastes from liquid media. Compared with other methods, adsorption allows for the most thorough purification of raw materials with relatively low cost (Dabrowski, 2001).

1.8 Ion exchange process

The removal of metal ions from the aqueous solutions is basically based on the ion exchange process. This process takes place at the solid/electrolyte solution interface and is referred to as ion exchange (Oscik, 1982c).The term "ion exchange" can be defined as the reversible interchange of ions between a liquid phase and solid, involving no radical change in the structure of the solid. Depending upon the ionic charge of the exchanging ions, the process maybe cation exchange or anion exchange (Mantell, 1951).

Cation exchange maybe carried out according to the salt cycle or the hydrogen cycle. In salt or sodium cycle, the adsorbent is employed in the sodium form and therefore exchanges sodium ions for other metals. Hydrogen cycle is defined as the complete course of cation exchange in which the adsorbent is employed in the hydrogen or free-acid form and therefore releases hydrogen ions to the solution and uptake the metallic ions. Both sodium and hydrogen cycles in the cation exchange process can be illustrated by Eqs. (1.1) and (1.2), respectively.

$$Ca^{2+} + Na_2Z \longleftrightarrow 2Na^{+} + CaZ \qquad (1.1)$$

$$2Na^{+} + H_2Z \longleftrightarrow 2H^{+} + Na_2Z \qquad (1.2)$$

where Z = cation exchanger