CHAPTER 1

INTRODUCTION

1.1 Rational and/or hypothesis

Aflatoxins contaminated in foods and animal feeds are a serious problem. The contamination in livestock feeds frequently results in poor growth and feed conversion efficiency, increased mortality rates, and a greater susceptibility to diseases [1]. They have been associated with various diseases for example aflatoxicosis, in livestock, domestic animals, and humans all over the world [2].

Aflatoxins are secondary fungal metabolites produced by *Aspergillus* species that have been detected before harvest, between harvesting and drying, during storage, after processing and manufacturing. Aflatoxins are known to be mutagenic, teratogenic and carcinogenic compounds [1, 3-4]. The ingestion of these toxins more a long period in very low concentrations may be highly hazardous. These compounds can enter the food chain, mainly, by intake through the dietary channel of humans and animals [5]. Among this group of toxins, aflatoxin B_1, the most toxic, is a potent carcinogen in many species and show a variety of biological effects [6-11]. Consequently, methods for reduction the toxicity of mycotoxins-containing foods and feeds are an urgent necessity. Various methods for reduce aflatoxins contaminated food and feeds have been reported in the literatures and recently reviewed. One of the strategies of current interest is the addition of adsorbent materials to the diet [3, 9, 12] that can bind and remove aflatoxins from aqueous solutions. Several studies showed

that a variety of adsorbents have high affinity for mycotoxins for examples, activated carbon, aluminosilicates (e.g. zeolites, hydrated sodium calcium aluminosilicate, clays), special polymer, and commercial adsorbents have been applied as toxin binders. An adsorption isotherm is a good tool for understanding the adsorption behavior of toxin on the adsorbent. Numerous models have been used in the literatures to describe the experimental data of adsorption isotherms. The Langmuir and Freundlich are the most frequently employed models. Therefore, these models were used to study adsorption characteristics.

In this study, four commercial adsorbents (i.e., the commercial toxin binder, the commercial bentonite, activated carbon, and the commercial zeolite) and two synthetic zeolites (i.e., synthetic Na-X zeolite and synthetic sodalite zeolite) were employed to binding aflatoxin B_1 *in vitro*. Various isotherm equations were applied to evaluate the adsorption efficiency and behavior of aflatoxin B_1 on these adsorbents.

1.2 Theory and literature review

1.2.1 Aflatoxins

Aflatoxins are natural mycotoxins, are secondary fungal metabolites mostly produced by *Aspergillus flavus* and *Aspergillus parasiticus* molds [1, 3-4, 8, 10-12, 14]. They were identified in the 1960s and comprise of a relative of toxic compounds. At least 20 different kinds of aflatoxins are constructed in nature with aflatoxin B_1 considered as the most toxic. The toxic effects contain acute hepatitis, immunosuppression, and hepatocellular carcinoma. Because of these toxic effects, the Food and Drug Administration allows the aflatoxin concentration in food and feed not exceeding 20 ppb (μg/kg) [15].

Aflatoxins can be detected before harvest, between harvesting and drying, during storage, after processing and manufacturing. They are frequently found in a variety of foods and feedstuffs such as corn, peanuts, breakfast cereals, cornmeal, cottonseeds, and various dairy products [1, 3, 10-12, 16].

1.2.1.1 Physical and chemical properties of aflatoxins

Aflatoxins, crystalline substances, can be dissolved in some polar organic solvent, for example, acetonitrile, methanol, chloroform, aqueous acetone, hexane- acetone-water azeotope and chloroform-methanol mixture. However, they cannot be dissolved in petroleum ether, ether, and hexane [17].

Crystalline aflatoxins are very stable in the absence of light and particularly UV radiation, even at temperatures above 100 ºC. Aflatoxin solution prepared in chloroform or benzene is stable for years if kept it in the dark and cold. The lactone ring makes them susceptible to alkaline hydrolysis, and processes involving ammonia or hypochlorite have been explored as means for their removal from food commodities, though questions concerning the toxicity of the breakdown products have controlled the use of this means to reduce aflatoxins from food and animal feeds. If alkaline treatment is soft, acidification will reverse the reaction to restructuring the original aflatoxin. Aflatoxin B_1 and G_1 are changed to aflatoxins B_{2a} and G_{2a} by acid catalytic addition of water across the double bond of the furan ring. Oxidizing reagents react, and the molecules lose their fluorescence properties [18].

Chemical structures of the six aflatoxins are shown in Figure 1.1. All aflatoxins are coumarin derivatives. They comprise a coumarin nucleus fused to a bifuran moiety. For aflatoxin B, a pentanone structure that is substituted in aflatoxin

G by a six-member lactone. Aflatoxin B_2 and aflatoxin G_2 are the dihydro-derivatives of aflatoxin B_1 and aflatoxin G_1 [7].

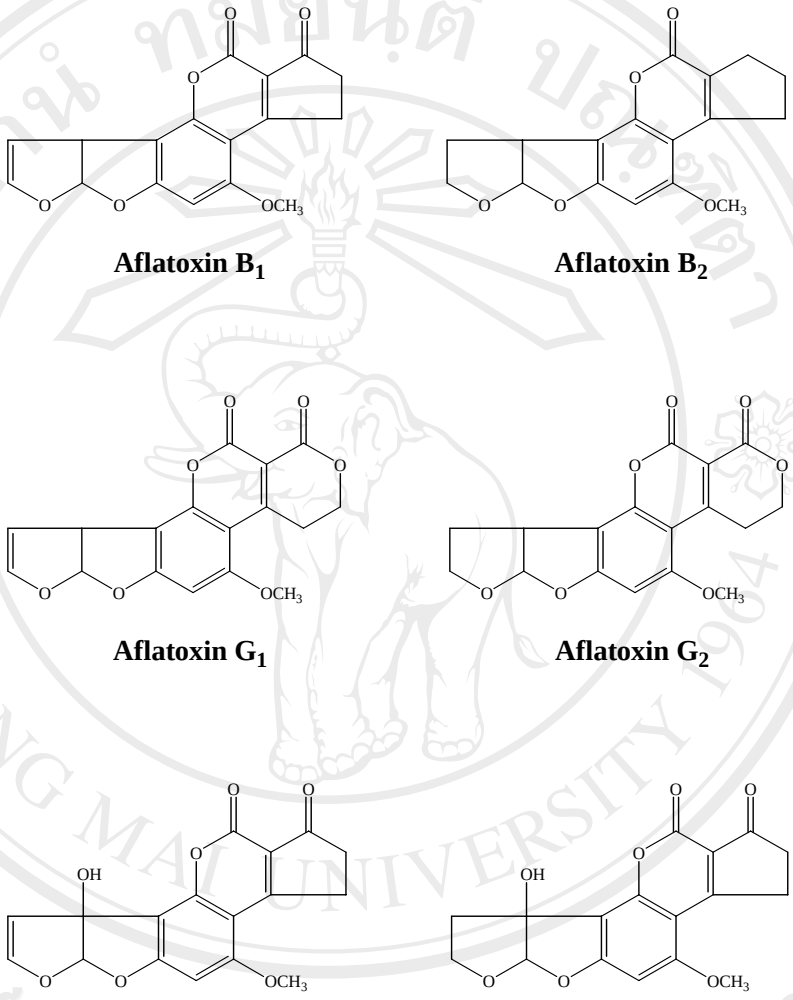

Figure 1.1 Chemical structures of aflatoxins

All aflatoxins are fluorescent under the ultraviolet light. For example aflatoxin B_1 and aflatoxin B_2 emit blue fluorescent, whereas aflatoxin G_1 and aflatoxin G_2 emit yellow-green fluorescent (turquoise), respectively. Some important physical and chemical properties of the aflatoxins are summarized in Table 1.1.

Additional, molecular size, light absorption and fluorescence properties of aflatoxins are presented in Table 1.2 to 1.3, respectively.

Table 1.1 Physical and chemical properties of aflatoxins [7, 17]

Aflatoxins	Molecular formula	Molecular weight	Melting Point (^{o}C)	Fluorescence under UV light
B_1	$C_{17}H_{12}O_6$	312	268-269	Blue
B_2	$C_{17}H_{14}O_6$	314	286-289	Blue
B_{2a}	$C_{17}H_{14}O_7$	330	240	Blue
M_1	$C_{17}H_{12}O_7$	328	299	Blue
M_2	$C_{17}H_{14}O_7$	330	293	Blue
M_{2a}	$C_{17}H_{14}O_8$	346	248	Blue
G_1	$C_{17}H_{12}O_7$	328	244-246	Turquoise
G_2	$C_{17}H_{14}O_7$	330	237-240	Turquoise
G_{2a}	$C_{17}H_{14}O_8$	346	190	Turquoise
B_3	$C_{16}H_{14}O_6$	302	217	Blue

Table 1.2 Molecular size of aflatoxins [19]

Aflatoxins	Molecular Size (Å)
B_1	5.18
B_2	5.18
G_1	6.50
G_2	6.50

Table 1.3 Light absorption and fluorescence properties of aflatoxins [7]

Aflatoxins	Ultraviolet absorption (ε)		Infrared absorption (cm^{-1})	Fluorescence emission (nm)
	265 nm	363 nm		
B_1	12960	20150	1760 1684 1632 1598	425
B_2	12320	23100	1760 1685 1625 1600	425
G_1	10670	17760	1760 1695 1630 1595	450
G_2	10020	20030	1760 1694 1627 1597	450
M_1	11000	21000	1760 1695 1628 1596	-

Aflatoxins B_1, B_2, G_1, and G_2, have high melting points, and they decompose to numerous products (Table 1.1). The derivative aflatoxins have also high melting points. In Table 1.3 presented light absorption and fluorescence properties of aflatoxins. The ultraviolet absorption spectra are similar, each show maxima at 223, 265 and 363 nm, respectively. For the fluorescence emission maximum, aflatoxin B_1 and aflatoxin B_2 emit florescent light at 425 nm, and aflatoxin G_1 and aflatoxin G_2 emit florescent light at 450 nm. The variation of emission property of all aflatoxins is useful in the evaluation of concentration of a compound by fluorescence technique [7, 20].

1.2.1.2 Occurrence of aflatoxins

Occurrence of aflatoxins in foods and animal feedstuffs are common found in many ways such as in raw agricultural products and in processed foods, these details are described below.

(i) Occurrence in raw agricultural products

Aflatoxins are detected rarely in milk, cheese, corn, peanuts, cottonseed, nuts, almonds, figs, spices, and a variety of other foods and feeds. Milk, eggs, and meat products from animal are sometimes contaminated due to the animal intake feed that are contaminated with aflatoxin. Nevertheless, the commodities with the highest danger of aflatoxin contamination are corn, peanuts, and cottonseed. They are frequently arising in crops in the field previous to harvest. Post harvest contamination can occur if crop drying is delayed, and during storage of the crop if water is allowed to exceed critical values for the mould growth. Insect or rodent infestations facilitate mould incursion of several stored produces.

Aflatoxin formation is also affected by associated growth of other moulds or microbes. For example, high temperatures, water stress, prolonged drought conditions, and high insect activity favour preharvest aflatoxin contamination of peanuts and corn, whereas warm temperatures and high humidity favour postharvest production of aflatoxins on corn and peanuts [2, 21].

(ii) Occurrence in processed foods

Aflatoxins are likely contaminated in several food products especially in corn. Corn is probably the product of greatest worldwide concern, it is grown in climates and is the staple food of many countries. Nevertheless, procedures used in the processing of corn can to decrease contamination of the significant food product. Since aflatoxins are moderate stable in most food processes, but they are unstable in processes for example those used in making tortillas that employ alkaline conditions or oxidizing steps. The contamination of aflatoxins in corn and cottonseed meal in

dairy rations have effected in aflatoxin M_1 contaminated milk and milk products, together with non-fat dry milk, cheese, and yogurt [21].

1.2.1.3 Toxicity of aflatoxins

Aflatoxins are both acutely and chronically toxic. Aflatoxin may cause acute toxicity with lethal effect when consume to large doses of aflatoxin, whereas exposure to small doses for delayed periods is carcinogenic. They are acutely toxic to most animal species. For chronic toxicity, is due to long-term exposure of moderate to low aflatoxin concentration. The symptoms comprise decrease in growth rate, lowered milk or egg production, pale liver, jaundice, swollen gall bladder, and immunosuppression [18, 22].

(i) Aflatoxicosis and animal health

Aflatoxicosis is primarily a hepatic disease. Aflatoxins can cause liver damage, reduction of milk and egg production, embryonic toxicity and recurrent infection because of immunity suppression. Various syndromes such as gastrointestinal dysfunction reduced feed utilization and efficiency, anemia, and jaundice are clinical signs of aflatoxicosis in animals. Nursing animals may be affected because of the change of aflatoxin B_1 to the metabolite aflatoxin M_1 excreted in milk of dairy cattle. Some environmental factors for example exposure concentration, exposure time, age, health, sex, and nutritional status of diet can influence the toxicity [2, 21].

(ii) Aflatoxins on human health

Humans can receive aflatoxins by consuming foods contaminated with products of fungal growth. Such exposure is difficult to avoid since fungal growth in foods is not simple to protect. The disease is characterized by vomiting, abdominal pain, pulmonary edema, convulsions, coma, and death with cerebral edema and fatty involvement of the liver, kidneys, and heart. Conditions increasing the probability of acute aflatoxicosis in humans include limited availability of food, environmental conditions that favor fungal development in crops and commodities, and lack of regulatory systems for aflatoxin monitoring and control. Additionally, the expression of aflatoxin-related diseases in humans may be influenced by factors for example sex, age, nutritional status, and/or concurrent exposure to other causative agents such as viral hepatitis or parasite infestation [2, 18, 22-23].

1.2.1.4 Detoxification of aflatoxins

Aflatoxins negatively affect the health of both humans and animals. To reduce or solve the problem of aflatoxins contamination, there have been numerous strategies for the reduction or inactivation of aflatoxins. They can be classified into chemical, biological, and physical ways [3, 8, 23-24].

A large number of chemicals can react with aflatoxins and change them to less toxic and mutagenic compounds. For example of these chemicals include formic acid, propionic acid, ammonium, sodium hydroxide, hydrogen peroxide, ozone, sodium hypochlorite, potassium permanganate, sodium borate, sodium bisulphate, calcium hydroxide using for detoxification of various mycotoxins. Chemical detoxification is very useful, although more accurate and faster, but not

always specific and does not meet the FAO requirements, since some compounds leave after their toxic metabolites and others reduce the nutritional value of the treated food and feed [16, 19, 25].

As biological way are not yet used in practice though the number of corresponding patents increases continuously. These methods comprise fermentation procedures with microorganisms, which are generally slow and incomplete [6]. Thus, the bioassay techniques are not suitable for routine screening purposes and their detection levels are not low sufficient [25].

For inactivation by physical way, there are many methods for reduced of aflatoxins such as extraction with solvents, inactivation by heat and adsorption from solution [24, 26] as described below.

(i) Extraction

Extraction with solvents, organic solvents such as ethanol, isopropanol and methoxymethane can eliminate all traces of aflatoxin from different types of food products. However, large-scale application of this method is limited by high cost of organic solvents. These organic solvents are not practical for industrial use since they themselves are not easily removed from the treated products [16].

(ii) Inactivation by heat

Aflatoxins are very stable to heat up to its melting point ranging from 237-306 ^{o}C. Common home cooking condition such as boiling and frying (approximate 150 ^{o}C) failed to destroy aflatoxin B_1. Temperatures more than 150 ^{o}C were important to reach partial destruction of the toxin. The amount of the destruction

attained was dependent on the initial level of contamination, heating temperature and time. Furthermore, the type of food and aflatoxin also influenced the degree of inactivation to achieve [24]. Nevertheless, in the presence of high humidity at high temperatures, destruction of aflatoxin over a period of time occurred. Such destruction can happen with either aflatoxin in oilseed meals, aflatoxin in roasted peanuts, or aflatoxin in aqueous solution. Even though the reaction products have not been examined in detail it seems likely that such treatment leads to opening of the lactone ring with the possibility of decarboxylation at elevated temperatures [17].

(iii) Adsorption

Nevertheless, most of these methods are impractical or potentially unsafe because of the formation of toxic residues of nutrient content, flavor, odor of the product. Therefore, practical and effective methods to decrease aflatoxins are required [23]. One of the most important approaches aimed at reducing the danger of aflatoxicosis is by adding of adsorbent materials to mycotoxin-contaminated diets [1, 3, 8, 12, 26-27]. Some adsorbent materials can bind and reduce mycotoxins from aqueous solutions. Examples are activated carbon, zeolite, hydrated sodium calcium aluminosilicate, bentonite, synthetic anion exchange resins and clays which have already established their efficiency. The zeolites are an adsorbent, commonly used in a variety of applications. They are aluminosilicate minerals and have a three-dimensional framework structure with interconnected cages and channels. Channels of zeolite filled with water, alkali, and alkaline earth exchangeable cations. When these channels are free of water, the adsorptive properties of the aluminosilicate are increased [12]. These properties enable zeolites to have many industrial and

agricultural applications including water and gas purification, hazardous waste cleanup, and as amendments to animal feeds and soils [29].

As clay mineral comprise of a complex of aluminosilicate structures. They are hydrous aluminium phyllosilicates, sometimes with variable amounts of iron, magnesium, alkali metals, alkaline earths and other cations. In each case, the interlayer can also contain water. The crystal structure is formed from a stack of layers interspaced with the interlayer [29]. Clays are widely applied in many fields of technology and science. The wide usefulness of clay is a result of their high specific surface area, high chemical and mechanism stability, and variety of surface and structural properties [30]. Examples are kaolinite, illite and montmorillonite, etc. Montmorillonite was also reportedly more effective for adsorbing aflatoxin B_1 rather than the other aflatoxins [9]. It was explained that montmorillonite was a layered silicate with the property of adsorbing organic substances either on its external surfaces or within its interlaminar spaces, by the interaction with or substitution of the exchange cations present in these spaces [3].

The applicability of aluminosilicates for the adsorption of mycotoxins has been reported in several literatures and recently reviews. Huwig *et al.* [6] used different adsorbents, including activated charcoal, aluminosilicates (i.e. zeolites, hydrated sodium calcium aluminosilicate, clays), and special polymers to bind mycotoxins by adding to mycotoxins-contaminated diet. It was found that aluminosilicates were the preferred adsorbents. In the early 1996s, Ramos and Hernandez [3] studied *in vitro* adsorption by used a montmorillonite silicate as adsorbent. They evaluated the affinity and capacity of montmorillonite to adsorb the four major aflatoxins. The data revealed that montmorillonite can adsorb aflatoxin B_1,

aflatoxin G_1, aflatoxin G_2, and aflatoxin B_2 of about 1000, 425-450, 230 and 200 µg/g of montmorillonite, respectively. Rosa *et al*. [4] studied the efficacy of bentonite to reduce toxic effect of aflatoxin. In vitro studies, bentonites have been shown to be effective for the adsorption of aflatoxin B_1 from aqueous solution [31] and they decreased the bioavailability of the aflatoxin in the gastrointestinal tracts of birds, when they were incorporated in the diets [32]. Since bentonite is a clay-based mineral mainly consisting of montmorillonite. Therefore, it has high effective for adsorb aflatoxin B_1 and commonly used as anticaking agent in animal feeds [11].

Also, Ramos *et al.* [12] reported that hydrated sodium calcium aluminosilicate was able to adsorb mycotoxins with high affinity. The addition of this compound to feedstuffs contaminated with aflatoxins showed a protective effect against the development of aflatoxicosis in farm animals. Similarly, Aly *et al.* [33] found that the commercially hydrated sodium calcium aluminosilicate had an excellent capability of adsorbing aflatoxin B_1 in an aqueous solution. Similar results were found by Grant *et al*. [15] with regard to use of hydrated sodium calcium aluminosilicate (phyllosilicate clay) for the adsorption of aflatoxin B_1. Hydrated sodium calcium aluminosilicate clay was shown to tightly bind aflatoxins in aqueous solutions. It obviously reduced the bioavailability of radiolabeled aflatoxins and greatly diminished aflatoxicosis in young animals, (i.e., rats, chickens, turkeys, lamb, and pigs) and reduced the level of aflatoxin M_1 in the milk from lactating dairy cattle and goats [10]. Pimpukdee *et al*. [1] used Novasil Plus, calcium montmorillonite clay, to bind aflatoxin B_1 *in vitro* and to prevent the onset of aflatoxicosis and vitamin A depletion in broiler chicks *in vivo*. *In vitro* results indicated that aflatoxin B_1 was tightly adsorbed onto the surface of Novasil Plus, which provided a high capacity and

high affinity. In addition to the *in vitro* studies, the effectiveness of Novasil Plus as an aflatoxin enterosorbent to attenuate the onset of aflatoxicosis in broiler chicks was determined at 3 different inclusion levels in the diet (0.5, 0.25 and 0.125%). Novasil Plus in the diet considerably protected chicks from the effects of high-level exposure to aflatoxins (i.e., 5 mg/kg) and preserved hepatic vitamin A levels, even at lower dietary intake of clay [31].

In addition, activated carbon is an adsorbent that widely used in many field. Activated carbon is obtained by pyrolysis and the activation of organic compounds of organic materials. It is a very porous non-soluble power with a high surface to mass ratio [6, 31]. The surface is non-polar or only slightly polar due to the surface oxide groups and inorganic impurities. Because of its large, accessible internal surface (and large pore volume), thus it can adsorbs more non-polar and weakly polar organic molecules than of other sorbents [34]. The intrinsic properties of the activated carbon are dependent on the raw material source. In recent years, Doll *et al.* [23] studied the efficacy of commercially available mycotoxin detoxifying agents and adsorbing substances as feed additives to detoxify deoxynivalenol and zearalenone *in vitro*. The *in vitro* model simulates the conditions of the porcine gastrointestinal tract. It was found that the activated carbon was able to bind both toxins whereas a modified aluminosilicate showed good adsorption abilities for zearalenone. These results were in conformity with the finding of Avantaggiato *et al.* [13] who investigated the *in vitro* adsorption of *Fusarium* mycotoxins on several adsorbing materials, including ion exchange resin, activated carbon, florisil, zeolite, bentonite, celite and commercial products. The results demonstrated that activated carbon had high ability of binding deoxynivalenol and nivalenol. Although, activated carbon can

adsorb most of the mycotoxins efficiently [6] but the *in vivo* studies were highly variable and resulted in either a complete lack of protection, or marginally effective protection. The results for activated carbon required more caution in their interpretation [35-36].

1.2.2 Adsorption studies

Adsorption is a surface phenomenon that is characterized by the concentration of a chemical species from its vapors phase or from a solution onto or near the surfaces or pore of a solid. This surface excess occurs in general when the attractive energy of a substance with the solid surface (i.e., the adhesive work) is greater than the cohesive energy of the substance itself. The adsorptive uptake is amplified if the solid material has a high surface area. If the adsorption occurs by London-van der Waals forces of the solid and adsorbate, it is called *physical adsorption*. If the forces leading to adsorption are related to chemical bonding forces, the adsorption is referred to as *chemical adsorption*. However, the distinction between physical adsorption and chemisorption is not always sharp. For example, the adsorption of polar vapors onto polar solids may fall under either classification, depending on the adsorption energy. From a thermodynamic point of view, the concentration of the substance from a dilute vapor phase or solution onto a solid surface corresponds to a reduction in freedom of motion of molecules and thereby to a loss in system enthalpy. As such, the adsorption process must be exothermic to the extent that the negative ΔH is greater in magnitude than the associated negative $T\Delta S$ to maintain a favourable free-energy driving force (i.e., for ΔG to be negative).

When a vapor is adsorbed onto a previously unoccupied solid surface or its pore space, the amount of the vapor adsorbed is proportional to the solid mass. The vapor uptake also depends on temperature (*T*), the equilibrium partial pressure of vapor (*P*), and the nature of the solid and vapor. For a vapor adsorbed on a solid at a fixed temperature, the adsorbed quantity per unit mass of the solid (*Q*) is then only a function of *P*. The relation between *Q* and *P* at a given temperature is called *adsorption isotherm*. *Q* is often presented as a function of the relative pressure, P/P^o, where *P* is normalized to the saturation vapor pressure (P^o) of the adsorbate at temperature *T*. The normalized isotherm is often more useful, as it enables one to assess readily the net adsorption heats and other characteristics of vapors over a range of temperatures [37]. Adsorption can be understood as the enrichment of one or more components in an interfacial layer. The solid is generally called the *adsorbent*, and the gas or vapour as the *adsorbate* and non-adsorbed gas is called the *adsorptive*. The fluid in the adsorbed state is called adsorbate [38-39].

The interaction between the surface and adsorbed species may be either chemical adsorption or physical adsorption. There are two principal modes of adsorption of molecules on surfaces.

(i) Physical adsorption (or physisorption)

Physical adsorption is the most general form of adsorption. The molecules are attracted by van der Waals forces, and connect themselves to the surface of the solid. The attraction is not fixed to a specific site and the adsorbate is relatively free to move on the surface. The attraction to the surface is weak but long ranged and the energy released upon accommodation to the surface is of the same order of magnitude as an

enthalpy of condensation (on the order of 20 kJ/mol). During the process of physisorption, the chemical identity of the adsorbate remains intact, that is no breakage of the covalent structure of the adsorbate takes place. Physisorption, to be a spontaneous thermodynamic process, must have a negative ΔG. Because translational degrees of freedom of the gas phase adsorbate are lost upon deposition onto the substrate, ΔS is negative for the process. Because $\Delta G = \Delta H - T\Delta S$, ΔH for physisorption must be exothermic [40].

Since heat of adsorption is small, the amount adsorbed only becomes substantial near or below the boiling point of the adsorbate; multilayer are easily formed at higher pressures. Similarly, the extent of adsorption on the surfaces of different solids under the same condition of temperature and pressure do not greatly vary [41].

(ii) Chemical adsorption (or chemisorption)

In chemical adsorption, the adsorbate attach to the solid by the formation of a chemical bond with the surface of the adsorbent. This interaction is greatly stronger than physisorption, and, in general, chemisorption has more stringent requirements for the compatibility of adsorbate and surface site than physisorption. The chemisorption may be stronger than the bonds internal to the free adsorbate that can effect in the dissociation of the adsorbate upon adsorption. There is a high degree of specificity and typically, a monolayer is formed [40].

Since chemisorption involves short-range chemical forces, it is normally limited to the monolayer. However, at higher gas pressures and at moderately low temperatures, the formation of a physisorbed or/and a weak chemisorbed layer of a

different character may, in some systems, proceed over the primary chemisorbed layer [41]. The difference between physical adsorption and chemical adsorptions is illustrated in Table 1.5.

Table 1.4 Characteristics associated with physical and chemical adsorption [42]

Criterion	Physical adsorption	Chemical adsorption
Heat of Adsorption / $kJmol^{-1}$	20 - 40 c.f. heats of liquefaction	> 80 c.f. bulk-phase chemical reactions
Rate of Adsorption (at 273 K)	Fast	Slow
Temperature Dependence of Uptake (with Increasing T)	Decreases	Increases
Desorption	Easy- by reduced pressure or increased temperature	Difficult - high temperature required to break bonds
Desorbed Species	Adsorbate unchanged	May be different to original adsorptive
Specificity	Non-specific	Very Specific
Monolayer Coverage	Mono or multilayer condition dependent	Monolayer only

If the adsorbent and adsorbate are contacted long enough equilibrium will be established between the amount of adsorbate adsorbed and the amount of adsorbate in solution. The equilibrium relationship is described by *isotherms*.

1.2.2.1 Adsorption isotherm

Adsorption is usually described through isotherms. Isotherm is functions which connect the amount of adsorbate on the adsorbent, with its pressure (if gas) or concentration (if liquid). An adsorption isotherm for a single gaseous adsorptive on a solid is the function that relates at constant temperature the amount of substance adsorbed at equilibrium to the pressure (or concentration) of the adsorptive in the gas phase. The surface excess amount rather than the amount adsorbed is the quantity accessible to experimental measurement, but, at lower pressures, the difference between the two quantities becomes negligible [43-45]. Several adsorption isotherms have established useful in understanding the process of adsorption.

The shape of the adsorption isotherm provides qualitative information about the adsorption process and the extent of the surface coverage by the adsorbate. Based upon an extensive literature survey, performed by Brunauer, Demming, Demming and Teller (BDDT), the IUPAC published in 1985 a classification of six adsorption isotherms [39]. The adsorption isotherms bases on IUPAC classification are shown in Figure 1.2.

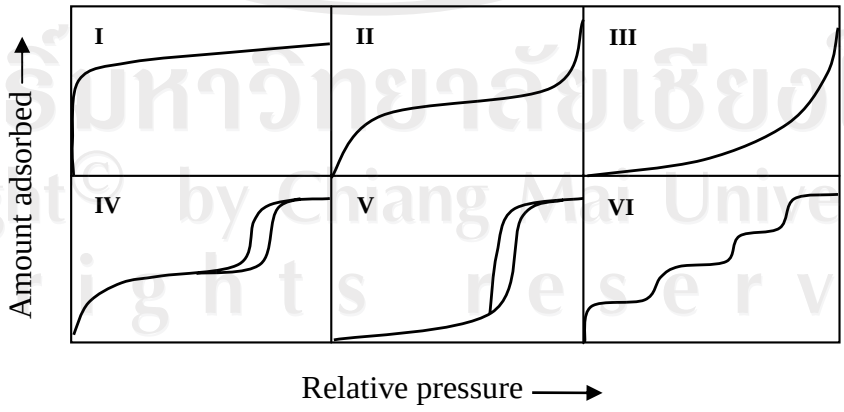

Figure 1.2 The six types of adsorption isotherms according to the classification of IUPAC [46]

Adsorption isotherms are typically nonlinear because of the energetic heterogeneity and the limited active sites on surface of the solid. The surface or porosity of the solid is generally the principle factor affecting the amount of vapor adsorption; hence, a powerful adsorbent must have a large surface area. Adsorption of a solute from solution is subject to competition by the solvent and other components in the solution. Therefore, a powerful adsorbent for single vapors is not necessarily a strong adsorbent for solutes from solution [37].

A number of adsorption isotherms have been recorded for vapor on a wide variety of solids [37], which figure as shown above. Type I isotherm approximates monomolecular adsorption and is frequently referred to as Langmuir type. This type of curve is obtained by low temperature adsorption of oxygen or nitrogen on certain charcoals and silica xerogels, because a monolayer saturates the surface or fills the pores. Chemisorption phenomena frequently produce this curve [47].

Type II isotherm is the most frequently encountered adsorption isotherm and is referred to as the Sigmoid or S-shape isotherm. The first portion of the curve up to relative pressure corresponds to monolayer. This is followed by a multilayer region. Its forms the basis of the Brunauer-Emmett-Teller (BET) model for surface-area determination of a solid from the assumed monolayer capacity [37, 47].

Type IV isotherm is similar to type II, except in that portion of the isotherm approaching 1.0 relative pressure. The sharp approach to the line corresponding to the saturation pressure shows a limited pore volume, because the diameter of the pores is only a small multiple of the diameter of the adsorbate molecules. This contour is common for many kinds of porous substrates.

Type III and Type V occur only when the force of monolayer adsorption are small. They are rarely encountered. A limited pore volume distinguishes the Type V isotherm, as compared with Type III.

Finally, the type VI isotherm that represented multilayer adsorption on a uniform, non-porous surface, particularly by spherically symmetrical, non-polar adsorptive. The sharpness of the steps depends on the homogeneity of the adsorbent surface and the temperature. Type VI isotherm is for example achieved with argon and krypton on graphitized carbon at liquid nitrogen temperature [38].

1.2.2.2. Isotherm equations

Many theories and models have been presented in the literatures to describe these different types of isotherms. Two isotherm equations, namely Langmuir and Freundlich models are the most frequently used to study adsorption behavior.

(i) Langmuir adsorption isotherm

The Langmuir isotherm was developed by Irving Langmuir in 1916 to describe the dependence of the surface coverage of an adsorbed gas on the pressure of the gas above the surface at a fixed temperature [49]. It is an empirical isotherm derived from a proposed kinetic mechanism [44]. The Langmuir isotherm is the simplest theoretical model for monolayer adsorption; hence this model has been widely used to characterize the adsorption behavior of solutes from aqueous solutions. The Langmuir model was originally developed to represent chemisorption on a set of distinct localized adsorption sites. The basic assumptions of this model is based on are

only monolayer adsorption takes place, adsorption is localized, all sites are energetically equivalent, and there is no interaction between molecules adsorbed on neighboring sites mean that the heat of adsorption is independent of surface coverage [34, 39, 41]. The classical Langmuir isotherm is giving by equation below:

$$q = Q_{\max}\left[\frac{K_d C_e}{1 + K_d C_e}\right] \quad (1)$$

Where q is the amount of aflatoxin adsorbed per unit of weight of adsorbent, Q_{max} is estimated maximum capacity, C_e is the concentration of unadsorbed aflatoxin at equilibrium, K_d is a constant that is related to the affinity constant. The values of Q_{max} and K_d in the Langmuir equation may be determined by rewriting the equation as:

$$\frac{C_e}{q} = \frac{C_e}{Q_{\max}} + \frac{1}{K_d Q_{\max}} \quad (2)$$

Equation (2) represents the linearized form of Langmuir equation. Thus, a linear plot of C_e/q versus C_e gives a slope of $1/Q_{max}$ and the intercept of $1/K_d Q_{max}$, respectively. From the slope and intercept, values of Q_{max} and K_d can be calculated, which curve shown in Figure 1.3.

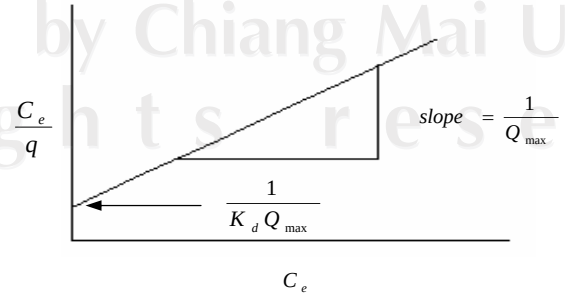

Figure 1.3 Characteristics of linearized Langmuir isotherm plot

(ii) Freundlich adsorption isotherm

Freundlich Isotherm is an equilibrium isotherm that is used most often in real world examples. The Freundlich equation was developed mainly to allow for an empirical account of the variation in adsorption heat with concentration of the adsorbate (vapor or solute) on an energetically heterogeneous surface [37]. This equation is the earliest known relationship describing the adsorption equation. It is based on adsorption onto a heterogeneous surface. The adsorption is not limited to a monomolecular layer, but can continue until a multimolecular layer of liquid covers the adsorbent surface. Many organic and inorganic follow this type of adsorption behavior [41].

The Freundlich equation is the summation of a distribution of Langmuir equation [39]. The Freundlich isotherm curves in the opposite way and is exponential in form and adsorption is described by the equation (3):

$$q = K_f C_e^{n^f} \tag{3}$$

It has the general form, where q is the amount of aflatoxin adsorbed per unit of mass of the adsorbent; C_e is the concentration of unadsorbed aflatoxin at equilibrium; K_f is the Freundlich constant, equal to the adsorption capacity constant and n^f is a constant related to the affinity. Taking the log of these terms, a straight line develops making easier to get the slope and intercept of the line. The linearized form is as follows:

$$\log q = \log K_f + n^f \log C_e \tag{4}$$

The constants of K_f and n^f are determined by plotting *log q* versus *log*

C_e, log K_f being the intercept and n^f the slope of the isotherm, as graph presented in Figure 1.4.

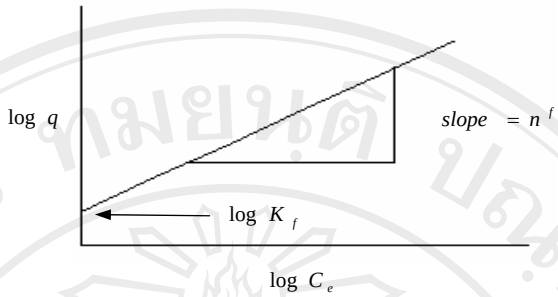

Figure 1.4 Characteristics of linearized Freundlich isotherm plot

The Freundlich equation with its adjustable parameters offers a simple mathematical tool rather than a physical model to account for the energetic heterogeneity of adsorption at different regions of the isotherm. For many applications, however, the Freundlich equation is quite mathematically convenient [37].

1.2.3 Determination of aflatoxins

Several methodologies for determination of aflatoxins have been developed. Methods routinely used today for aflatoxins base on UV/VIS spectrophotometry [1, 4, 15, 32, 35, 37], high performance liquid chromatography (HPLC) [4-5, 32, 49-52], thin-layer chromatography (TLC) [53-55], and enzymelinked immunosorbent assay (ELISA) [7, 50].

1.2.3.1 UV-VIS spectrophotometry

UV-VIS spectrophotometry technique for determination aflatoxins have been reported in several literatures [1, 4, 15, 32, 35, 37]. UV-VIS absorption

spectroscopy is usually applied to molecules or inorganic complexes in solution. They can be used to determine the concentration of an analyte in solution by measuring the absorbance at some wavelength and applying the Beer-Lambert Law [56-57]. The Beer-Lambert law states that the absorbance of a solution is directly proportional to the solution's concentration. Aflatoxin B_1 can absorb UV light at 223, 265, and 362 nm with maximum absorption at 362 nm [24]. Therefore, the aflatoxins concentrations in each supernatant could be determined by UV-VIS spectrophotometry. The percentage of aflatoxins adsorption was calculated as difference between the added amount and the aflatoxins remaining in the supernatant after incubation.

1.2.3.2 High performance liquid chromatography

High performance liquid chromatography (HPLC) is currently the technique of choice for aflatoxins separation [53] because of their sensitivity, enhanced accuracy, and they can be applied to a number of mycotoxins comprise aflatoxins B_1, B_2, G_1, and G_2 [25]. Liquid chromatography (LC) is similar to thin layer chromatography (TLC) in many respects, including analyte application, mobile phase and stationary phase. LC and TLC complement each other. For an analyst to use TLC for beginning work to optimize LC separation conditions is not unusual. Liquid chromatography methods for the determination of aflatoxins in foods consist of normal-phase LC (NPLC), reversed-phase LC (RPLC) with pre- or before-column derivatization (BCD), RPLC followed by post column derivatization (PCD), and RPLC with electrochemical detection [21]. Also, several mass spectrometry (MS) based methods for aflatoxin determination have been published. To confirmation of

aflatoxin contamination, a mass-spectrometric (MS) technique is the most suitable [32, 49-52].

In the analytical procedures of aflatoxin investigation by HPLC, there are three steps including extraction, clean up and quantitative determination. LC methods for determining aflatoxins in foodstuff are difficult and time consuming. Frequently, these techniques need knowledge and skill of chromatographic techniques to solve separation and interference problems. Additionally, many laboratories used enzyme-linked immunosorbent assay (ELISA) testing for screening. ELISA analysis is suitable for simultaneous determination of contaminants in a large number of samples with relatively low cost and short time. Nevertheless, it is not convenient for quantification of contaminants since it can be influenced by matrix effect of samples and has the possibility to overestimate the contaminants at very low concentration [5, 21, 50].

In this work, UV-VIS spectrophotometry was applied to determine the amount of aflatoxin B_1 adsorbed on the adsorbent.

1.3 Research objectives

(1) To study the isothermal adsorption of aflatoxin B_1 on some commercial adsorbents (the commercial toxin binder, the commercial bentonite, activated carbon, and the commercial zeolite) and synthetic zeolites (synthetic Na-X zeolite and synthetic sodalite zeolite)

(2) To evaluate the capacity and affinity constants of the adsorbent materials in adsorbing the aflatoxin B_1 using various isotherms

(3) To compare the adsorption efficiency of commercial and synthetic adsorbents in binding aflatoxin B_1 from aqueous solutions