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ADSORPTION OF GALLIC ACID IN AQUEOUS SOLUTION BY AMBERLITE XAD-7

Dissertation submitted in partial fulfillment for the Degree of Bachelor of Science (Health) in Forensic Science

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CERTIFICATE

This is to certify that the dissertation entitled

'Adsorption of gallic acid in aqueous solution by Amberlite XAD-7'

Is a bonafide record of research work done by

Mr. Abdul Muiz bin Aziz

during the period of 16th December to 30th April 2009

under my supervision.

Dedicated to my dearly-loved mother

Hajah Aminah binti Yaakob

and my late beloved father

Aziz bin Yusoff

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ABSTRACT

The adsorption behaviour of gallic acid onto XAD-7 was studied to determine the effects of pH, contact time, temperature, concentration of gallic acid and amount of XAD-7. The adsorption capacities were strongly influenced by the solution pH where the optimum working pH was found to be at pH 2.0. The shaking time (45 min) was found suitable for maximum adsorption. The batch equilibrium data were correlated to Langmuir and Freundlich isotherm and the data fitted better to the Langmuir isotherm. The thermodynamic parameters were calculated and the corresponding value showed that the adsorption of gallic acid onto XAD-7 was spontaneous, entropy-driven and endothermic.

INTRODUCTION

Polyphenols or phenolic compounds are a large family of metabolic compounds that occur naturally in a wide variety of plant foods and materials. Phenolic compounds are considered as secondary metabolites. Each phenolic compound must have a phenolic structure which is a hydroxyl group on an aromatic ring. From this structure, larger and interesting molecules are formed such as anthocyanins, coumarins, phenylpropamides flavonoids, tannins and lignin.

Other than providing humans with exceptional benefits, phenolic compounds also contribute many advantages to plants. Among others are defending against herbivores and pathogens, absorbing light, attracting pollinators, reducing the growth of competitive plants and promoting symbiotic relationship with nitrogen-fixing bacteria (Wildman, 2001). The pathways that form phenolic acids are biosynthetic because they occur in living cells. There are two pathways that can yield phenolic compounds which are the shikimic acid pathway and malonic acid pathway. Shikimic acid pathway is more prominent in higher plants while the malonic acid pathway is predominant in lower plants, fungi and bacteria.

They are well known for their antioxidant properties. Antioxidants can protect the cells from oxidative damage diseases such as cancer, coronary heart disease, and stroke. There are about 8000 naturally occurring phenolic compounds where they can be divided into phenolic acids and flavonoids (Luthria *et al.*, 2006).

Phenolic compounds can be classified into smaller subgroups which are phenolic acids, flavonoids, isoflavonoids, lignins, stilbenes, and complex phenolic polymers (Vuorela, 2005). They have been demonstrated in abundant studies as having antioxidant, anticarinogenic and antimicrobial properties (Yeh & Yen, 2006; Wojdylo & Ozmainski, 2007; Kim *et al.*, 2008; Teixeira *et al.*, 2008; Subhasree *et al.*, 2008; Heredia *et al.*, 2009; Kathirvel *et al.*, 2009). These beneficial properties were proven to be present in fruits, vegetables and plant materials that grow in their native country.

The content of total phenolic acids in fruits can range from 0 to 103mg/100g in rowanberry. Other excellent fruits with high phenolic acid contents are chokeberry (96 mg/100 g), blueberry (85 mg/100 g), sweet rowanberry (75 mg/100 g), and saskatoon berry (59 mg/100 g). Other fruits include dark plum, cherry and apples with a rough amount of 28 mg/100g phenolic acids. In drinks, coffee contains 97 mg/100 g while green and black teas have about 30-36 mg/100 g phenolic acids. Here caffeic acid is the highest phenolic acid content (Sahelian, 2006).

Phenolics also contribute to the flavor, taste, and medicinal properties of spices. Most spices contain phenolic acids such as tannic, gallic, caffeic, cinnamic, chlorogenic, ferulic and vanillic acids. A high amount of tannic and gallic acids are found in black mustard and clove. Caffeic, chlorogenic and ferulic acids are found in a good amount in cumin. Vanillic and cinnamic acids are found in onion seeds. Various studies have been done to exhibit the antioxidant properties of phenolic acids. For example, antioxidant properties of bananas, seedless guava and pineapple in Malaysia has been proven (Alothman et al. 2009)

Phenolics also exhibit good antioxidants as well as antimicrobial activities as revealed by crude sorghum extract in Korea (Young Kil *et al.*, 2009).

Another superb benefit of phenolics is decreasing the fat content in body. Pancreatic lipase is a digestive enzyme that splits triglycerides into absorbable glycerol and fatty acids thus increasing total body fat content. Polyphenols have been found to inhibit the action of this enzyme. The polyphenols were found in various types of berries like blueberries, lingonberries, cloudberry, strawberry and raspberry (McDougall *et al.*, 2009).

Gallic acid is an organic acid as well as belonging to the subgroup of phenolic acid. Its chemical name is 3,4,5-trihydroxybenzoic acid. It can be found in a variety of plants, nuts, grains, fruits and tea leaves. Its chemical formula is $C_6H_2(OH)_3COOH$. Gallic acid contains carboxyl group and a phenolic –OH group where its H can be used to bond to various compounds (Saha *et al.*, 2009).

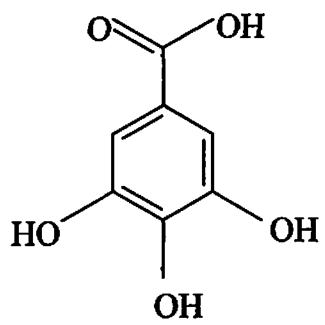

Fig. 1. Structure of gallic acid.

Gallic acid has a molar mass of 170.12g/mol and is soluble in water. Gallic acid is present in plants in free form as well as tannins which are phenolic compounds found mostly in the bark of trees commonly in the oak tree (Hagerman, 2002). In tannin form, gallic acid is called gallotannins and hydrolysis of this compound yields gallic acid (Galvez, 1997). Salts and acids of gallic acid is called gallates.

Research shows that a wide variety of berries contain gallic acid. Among others are blueberries (Zeng & Wang, 2003; Sellapan *et. al*, 2002), blackberries, linganberries and cranberries (Sellapan *et. al*, 2002), bayberries (Fang et al, 2006), grapes, black currant, strawberries and raspberries (Hakkinen, 1999). Other plant foods and fruits that contain gallic acid include chest nuts of the Portuguese variety (De Vasconselos *et al.*, 2007), legumes (Galvez, 1997), pomegranate and lemon juice (Gonzales-Molina *et al.*, 2008), mangoes (Kim *et al.*, 2009) and in thyme as antioxidants.

However, the quality and quantity of gallic acid and other polyphenols in these fruits and plant materials decrease with time during storage and heat treatment. Therefore, the fresher the produce the more excellent they are in containing these great nutrients that harbor excellent medicinal benefits (Heredia *et al.*, 2008; Cerretani *et al.*, 2008; Kim *et al.*, 2009). The total content of phenolics may also decrease as the fruit ripens and becomes quite low when the fruit reaches the later stage of ripening (Righetto *et al.*, 2005).

Gallic acid has been described as having excellent antioxidant properties (Zeng & Wang, 2003; Sellapan *et. al*, 2002; Liu *et. al*, 2005; Moein *et. al*, 2007). Because of this, gallic acid has been manipulated and used in many areas of research and also in many food and drink manufacturers. In one study, gallic acid has been demonstrated as being a brilliant antioxidant in inhibiting the peroxidation of lipids in daily diet which may lead to atherosclerosis, ageing and cancer (Rafiquzzaman *et al.*, 2006). It also possess antimutagenic and anticancerogenic properties (Rawel *et al.*, 2005)

The presence of free radicals and certain chemical reaction may lead to protein oxidation and DNA damage in living cells (Morrissey & O'Brien, 1998). In order to reduce such aversive health effects that may lead to chronic diseases, whole grains, fruits and vegetables must be included in the everyday diet (Hu, 2002).

Antioxidants are substances which inhibit or delay oxidative processes. The oxidative process is the work of free radicals which are electrically charged molecules having unpaired electrons and therefore an affinity to capture electrons from other substances. This results in the neutralization of the free radical but causes the production of another free radical leading to a chain reaction. Within a few seconds thousands of free radicals are produced. As a result of such oxidation, cells become damaged leading to aging, cancer, cardiovascular disease, cataracts, immune system breakdown and brain dysfunction (Moein *et al.*, 2006).

Phytochemicals are non-nutrient compounds found in plant derived foods that have biological activities in the body. There are thousands of phytochemicals available but not all have been researched. Phytochemicals are well known for their antioxidant properties and gallic acid and phenolic acids are examples of phytochemicals. These phtochemicals minimize damage by limiting free-radical formation, destroying free radicals or their precursors, stimulating anti oxidant enzyme activity, repairing oxidative damage and stimulating repair enzyme activity. Examples of fruits which contain phytochemicals include soybeans, tomatoes, guava, papaya, pink grapefruits and watermelon.

Phenolic acids are phytochemicals and their effects in the body as antioxidants involve triggering enzyme production to make carcinogens water soluble and thus helping the excretion of the carcinogens (Rhigetto *et al.*, 2005). They are abundant in coffee beans, apples, blueberries, cherries, grapes, oranges, pears, prunes, oats, potatoes and soybeans (Whitney *et al.*, 1990)

Foods like fish and meat contain lipids that may undergo oxidation thus deteriorating the quality of such food in terms of odour, flavor, texture, colour and nutritional value. Therefore, antioxidants are added to these foods so that they may not undergo lipid oxidation. Since plants contain a broad variety of antioxidants in the form of phenolic acids, they are used by food manufacturers. Furthermore, they are more widely accepted by the due to their natural origins (Kathirvel *et al.*, 2008).

Gallic acid has also been proven to be good antimicrobial and enzymatic inhibitors for plant pathogenic bacterias which causes crops to rot. The bacterias are the *Erwinia* species and *Pectobacterium chrysanhemi* which secretes protease and pectate lyase causing the maceration of plants. The mechanism to which gallic acid acts in inhibiting the bacterial activities is by the reduction of the availability of the substrates by combination of polysaccharides and proteins (Zaidi *et al.*, 2008).

LITERATURE REVIEW

The process of adsorption involves the accumulation of gas or liquid solute on the surface of a solid or liquid forming a film of molecules or atoms which is called the adsorbate. Adsorption is applied and utilized widely in industrial applications for example activated charcoal, synthetic resins and water and air purification systems. The bonding of the adsorbent to the adsorbate species is due to van der Waals forces and covalent bonding (Uner, 1998).

Some characteristics that must be possessed by adsorbents are the ability to withstand high temperatures and abrasive conditions. They must also possess small pore diameters so that the adsorbents' surface area is increased resulting in higher adsorptive capabilities. There are three main classes of industrial adsorbents which are oxygen-containing compounds like silica gel, carbon based compounds like activated carbons and finally polymer based compound like the Amberlite XAD variety (Cussler, 1997). These adsorbents are mainly used to adsorb pollutants like anionic dyes, trace metals, polyaromatic hydrocarbons, phenols and many more from an array of environmental samples.

There are many adsorbents designed by researchers whether using materials from natural resources or adsorbents that were synthesized chemically. Examples of adsorbents from natural resources include olive pomace (Francesca *et al.*, 2007), durian rind pectin (Wong *et al.*,2008), activated sludge from the biological treatment facilities in the treatment plants (Al-Qodah, 2005), tamarind fruit shells (Popuri *et al.*, 2007), oat hulls (Chuang *et al.*, 2005), jute stick and leaf, sugarcane, lily leaf, fish ayes powder, egg's skull, fern and water hyacinth root (Islam *et al.*, 2007), agricultural by-products (Wang, 2008) and many more. The list just goes on as the possibilities of materials that can be used are limitless. These adsorbents are called biosorbents and are mainly developed into activated carbon. These type of adsorbents are preferred due to their eco-friendly nature, effective and low-cost development (Al-Qodah, 2005). The mechanism in which adsorption occurs is through the deposition of the adsorbate species on the surface of biosorbent through different sorption processes of ion exchange, complexation, chelation, microprecipitation, etcetera.

There are many factors that may affect the process of biosorption including temperature where the range of 20-35 °C has found to be optimum for most biosorbents (Aksu *et al.*, 1992). The pH of the environment is the most important determinant in the biosorptive process because it affects the solution chemistry of the sorbate, the activity of the functional groups in the adsorbant and the competition of the sorbate ions (Friis *et al.*, 1998). The concentration of the adsorbent also plays a role in the efficiency of the adsorbance as demonstrated in many studies where lower biomass concentration results in an increase of the sorbate species uptake by the adsorbents (Sudha & Abrahan, 2003).

Other than biosorbents are synthetic adsorbents which are mainly synthesized by companies. Examples of synthetic adsorbants include synthetic byrnessite or mangan dioxide which was used to adsorb arsenic in drinking water (Manning *et al.*, 2002). Another synthetic adsorbant that are used by researchers is synthetic zeolites which was manufactured by chemical companies (Chutia *et al.*,2009).

However, synthetic adsorbents which are popular among researchers are the Amberlite XAD resins. Amberlite XAD resins have been used widely by scientists for adsorbing a wide variety of species, the most common being environmental pollutants. Amberlite XADs are manufactured by the company Rohm and Haas and act via solid phase extraction. This company has produced an extensive range of XADs where each type has certain characteristics suitable for certain sorbate species.

Polymeric adsorbents like the Amberlite XAD variety possess highly porous structures with internal surfaces that are able to adsorb then desorb a widespread range of species depending on the environment. In polar solvents like water, the adsorbents display non-polar or hydrophobic behaviour hence capable of adsorbing organic species that are soluble in water. In cases of non-polar solvents like hydrocarbons the polymeric adsorbents may exhibit slightly polar or hydrophilic behaviour thus adsorbing species containing some degree of polarity. This type of behaviour is prominent in acrylic adsorbents like XAD-7.

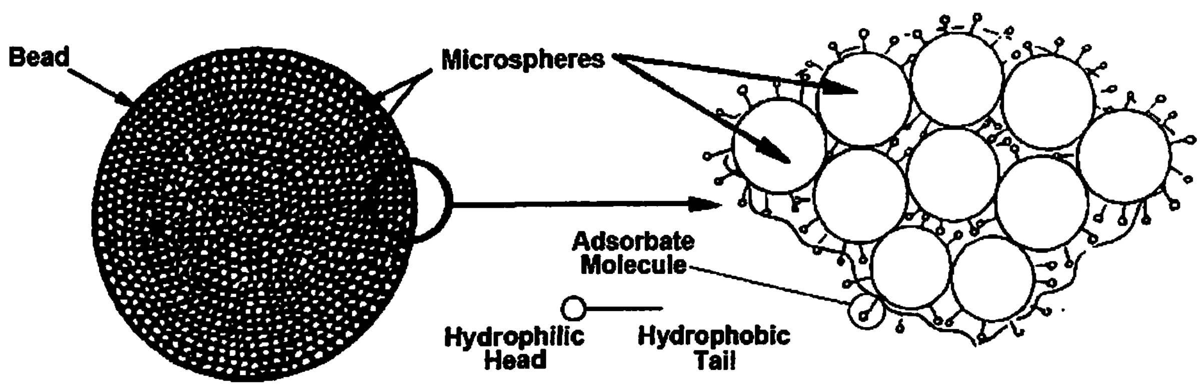

Fig. 2. Behaviour of Amberlite XAD bead

There are also an assortment of other Amberlite XAD polymeric resins manufactured by Rohm and Haas company which are XAD-2, XAD-4, XAD-16,XAD-1180, XAD-1600, XAD761, etc. These resins are divided into two main groups which are the polystyrene-vynil based resins like the XAD-1, XAD-2, XAD-16, XAD-1180 and XAD-2000 resins and the polyacrylic acid ester based resins which consists of XAD-7, XAD-8 and XAD-11 (Rohm and Haas company brochure).

The Amberlite XAD resins or polymeric adsorbents that can be used to adsorb phenols, antibiotics, chlorinated pesticides, various aromatic and nitrogen compounds from aqueous streams, hydrogen peroxide and many more. An example of Amberlite XAD being used include XAD-2000 where the resins were placed in a column in order to adsorb trace metals like Co, Ni, Cu, and Cd from water and plant samples (Duran *et al.*, 2008). In another study, polyaromatic hydrocarbons (PAH) which are carcinogenic were passed through an adsorbent tube containing XAD-2 (Wei, 1995). XAD-2 was also demonstrated to be able to adsorb effectively trace amounts of Cu(II) and Zn(II) ions (Ferreira *et al.*, 1998).

XAD-2010 was utilized in one study to adsorb Mn, Fe, Co, Ni, Cu, Zn, Cd and Pb from water and found to be efficient. Here, the samples were passed through a column containing XAD-2010 so that the metals will be chelated onto the resins prior to atomic spectrometric analysis (Gundogdu, 2007). XAD-4 was also used to adsorb organophosphorus compound or OCP from the atmosphere (Nerin *et al.*, 1995).

According to the company Rohm and Haas's brochure, XAD-7 is hydrophilic and made of acrylic ester, has the particle mesh size of 20 to 60, a surface area of $450m^2/g$ and average pore diameter of 90 A. The brochure also stated that XAD-7 is ideal for the adsorption of ketones, esters and aliphatic compounds from polar solvents. It is also suitable for the removal of polar compounds from non-aqueous solvents.

XAD-7 has been demonstrated to be very efficient in adsorbing a variety of species. However, to date there are no studies reported that have used XAD-7 to adsorb galic Acid. A study was conducted to remove phenols from wastewater where XAD-7 was placed in a column and the wastewater passed through the column and found that XAD-7 was a good adsorbent for phenols in wastewater (Kujawski *et al.*, 2004).

In another study, XAD-7 was used to extract antioxidant phenolic compounds from mountain papaya *Vasconcellea pubescens* grown in Chile. Here, the fruit extract in aqueous from was passed through a column containg the resins and then eluted with methanol. As a result two phenolic compounds were found and identified via HPLC which were quercetin glycosides rutin and manghaslin (Simirgiotis *et al.*, 2009).

In order for the adsorption to be optimized, certain parameters must be tested in order to find the recovery efficiency of the sorbate. The parameters include the pH of the sorbate, contact time of sorbate and sorbent, temperature and concentration. However, the factors that play a major role in determining the adsorbance efficiency are pH and contact time. The pH dependence is due to the type and ionic states of the functional groups present in the adsorbent as well as the chemistry of the adsorbate species in aqueous solution. At low pH, the adsorbate becomes protonated and will favour the uptake of the sorbate species in anionic form. With an increase in pH, the sorbent gradually becomes deprotonated resulting in adsorption of cationic ions. Also at low pH, there is an abundant of H+ ions hence cationic species must compete with the H+ ions to be sorbed onto the resins. On the contrary, in solutions of high pH where the anionic species must compete with high levels of OH- ions for sorption onto the resins (Quintelas *et al.*, 2007; El-Sofany, 2007; Krishnani *et al.*, 2007). Therefore it shall depend on the sorbate species desired. The contact time also plays a role where after maximum adsorption has occurred equilibrium and has been reached. At this point, the resins cannot adsorb anymore sorbate species (Quintelas *et al.*, 2007).

RESEARCH OBJECTIVES

1. To determine the effects of pH, shaking time, amount of adsorbent, concentration of adsorbate and temperature on the adsorption of gallic acid onto XAD-7.

2. To assess the thermodynamics of the adsorption process.

3. To apply the data obtained onto two isotherm models and determining the best fit.

MATERIALS AND METHODS

Chemicals and Reagents

Spectroscopic or chromatographic grade chemicals and reagents were obtained from Merck/ Fluka (Germany) and Sigma (Germany). Gallic acid standard was purchased from ACROS Organics (USA). The Amberlite XAD-7HP was purchased from Sigma Aldrich (Germany). The chemicals were used as received or otherwise stated.

Glassware

All the glass ware used was soaked overnight in a 1:2 v/v mixture of HCl/HNO_3, washed thoroughly using distilled water and dried in the oven at 105°C.

Purification of Amberlite XAD-7 Resins

The Amberlite XAD-7 resin was purified by washing 20 g of the resin with a 1: 5 v/ v mixture of deionized H_2O and MeOH and dried at 50 °C.

Preparation of Gallic Acid Solution

Stock standard solution of gallic acid (100 ppm) was prepared by dissolving 25.0 mg of gallic acid in 250 ml double distilled water to test the adsorption ability of XAD-7. This solution was used in 25 mL portions for the batch equilibrium studies.

UV-Visible Spectrophotometer

A cary 100 double-beam UV-visible spectrophotometer was used to record the spectrum of gallic acid at 210 nm. The instrument was calibrated prior to use to check its wavelength accuracy. The cuvettes were washed before running the samples to remove traces of impurities and chemicals from previous analyses. The UV-Visible spectrum of the 100 ppm gallic acid solution was measured.

Batch Adsorption Experiments

The batch adsorption experiments were carried out following the literature (Bilgili, 2006; El-Sofany, 2008). First, the pH of gallic acid solution was measured using UV-Vis for the absorbance value. Later, the pH of acid solution was adjusted by using pH meter to pH 2.0, pH 4.0, pH 6.0 and pH 8.0. Hydrochloric acid and sodium hydroxide was selected as acid and alkali respectively to adjust the pH of the acid solution. The absorbance of the solution was then measured before shaking together with the XAD-7 and noted as the initial concentration, C_0.

The speed of the Heidolph Instruments Rotamax 120 shaker was set to 120 rpm for the whole experiment. Before shaking, an accurately measured amount of 0.5 g XAD-7 was added each into 25 mL of acid solution contained in a 100 mL conical flask. The contact time of XAD-7 with the acid solution was 15 min, 30 min, 45 min, 60 min, 75 min, 90 min, 105 min and 120 min in the room temperature. At each interval, the XAD-7 was filtered out to collect only the acid sample to measure the amount of acid left inside the solution. This measurement will be analyzed by UV-visible spectrophotometer to find out the amount of acid adsorbed by the XAD-7. This was repeated for pH 2.0, 4.0, 6.0 and 8.0.

After that the optimum pH and shaking time were selected and used as constants for the next three parameters which are concentration of gallic acid solution, amount of