CHAPTER 1

Introduction: The Purpose and Scope of Study

SUMMARY

This chapter introduces the study. Occurrence of soluble pharmaceutically important compounds in plant products is abundant but their separation and purification particularly in maintaining the original value and also minimizing the residual effects because of predominant use of various chemical extraction methods is often difficult. Separation of pharmaceutically important plant products and their need of study to explore new purification methods to overcome the problems are discussed. Two pharmaceutically significant water soluble plant products viz. caffeine and ginsenoside are selected and taken as standard representative compound for undertaking the present study. An adsorptive separation technique, which is based on polymeric resins, is used as a potential solution to the separation difficulty. The scope of the study, its background and objective are also presented in this chapter. The structure and properties of the selected compounds along with those of the polymeric resins and their advantage of use in separation and purification processes are presented and discussed. Chapter 1 also includes an overview of the adsorptive separation process and its significance and importance with reference to separation and purification of high valued plant products from aqueous solutions.
1.1. Introduction

1.1.1 Background

The pharmaceutically active compounds in the plant species which produces secondary metabolites like *triperpenoid saponins* and *alkaloids* are the focus of much attention. It has been claimed that these complex biomolecules from plant origin have many beneficial bioactive effects on human health. Some of the important compounds found in high relative yields with cultured plant cells\(^1\) are *ginsenoside* (*Panax quinquefolium*, *Panax Ginseng*), *Berberine* (*Berberis aquifolium*, *Coptis japonica*, *Hydrastis Canadensis*), *Caffeine* (*Camellia sinensis*, *Thea sinensis L*, *coffea Arabica*), *Ajmalacine* (*Roots of Catharanthus Roseus*) etc.

In addition to cultivation and propagation of the said pharmaceutically active plants, plant cell cultures are also considered as an alternative source to whole plant for the efficient production of the valuable secondary metabolites. This is an active current area of research and development around the world, possessing great potential for industrial production of biomedicines. A highly efficient extraction and purification technique for isolation of the bioactive molecules from these plant products and their cultured cell mass is therefore very important to reduce its cost and to have wide pharmaceutical applications. The traditional methods of solvent extraction of plant materials are mostly based on the choice of solvents and use of heat and/or mixing to increase the solubility of and the rate of mass transfer\(^2\). Soxhlet extraction involves solid-liquid contact for removal of one or more compounds from a solid by dissolution into a refluxing
liquid phase. Its advantage is to repeatedly bring the sample into contact with fresh portions of the solvent, which prevents the possibility of the solvent becoming saturated with the extractable material and thus enhances the removal of the analytes from the matrix. Moreover, because of the system-temperature remaining slightly higher than the boiling point of the solvent, the excess energy in the form of heat helps to increase the extraction kinetics of the system\textsuperscript{3}. Heat reflux system of extraction is a solid-liquid extraction, which is accomplished by allowing hot solvent to leach out the compounds from the solid tissue. The technique allows extraction of the solid at an elevated temperature without loss of solvent under evaporation.

Liquid-liquid extraction is usually employed to separate biomolecules from the plant extracts. However, with the discovery of trace important bioactive compounds and development of plant cell culture technologies for mass production of the pharmaceutically important secondary metabolite compounds, it is essential to develop alternative and economically viable separation processes, since the liquid-liquid extraction is less favourable for many recovery processes from crude plant extracts\textsuperscript{4}.

Different extraction methods including conventional organic solvent, heat reflux, Ultrasound assisted and microwave assisted extraction methods were attempted for efficient extraction of \textit{bioactive saponins} from the cell mass obtained in bioreactor cultures. Ultrasound assisted extraction enhances both solvent penetration into the plant materials and the release of intracellular product
by disruption of the cell walls mainly due to the mechanical effects of the acoustic cavitations\textsuperscript{5}.

Another relatively new method of extraction is the micro-wave assisted extraction which combines microwave with the traditional method of solvent extraction. Microwave assisted extraction depends on the dielectric susceptibility of both solvent and the cell matrix. Microwave energy causes motion by migration of ions and rotation of dipoles\textsuperscript{6} and therefore depends on the presence of polar molecules or ionic species. This is relatively new technique of secondary metabolite extraction from plant materials and yet to find broad commercial applications.

All these extraction methods have their merits and demerits from the viewpoint of applicability and large scale economic extraction. There is therefore wide scope of making proper scientific study for upgrading the \textit{Alkaloids} and \textit{Saponin} extraction process and technology for efficient and economic use.

Adsorption chromatography is a recommended process for extraction and purification of synthetic compounds and the adsorbents known from literature are activated carbon, molecular sieves and non-ionic/ ion exchange resins. Due to its high concentrating factor, adsorptive separation is considered to be better method for biomolecule separations\textsuperscript{7-10}. Though much work has been done to improve the separation of various compounds by analytical liquid chromatography, systematic investigations on biomolecules like the \textit{Alkaloids} and \textit{Saponin} extraction have not been made. There is therefore limited knowledge on the molecular details of the
adsorption process for many biomolecules including studies on technological perspectives.

1.1.2 Objectives

Adsorptive interaction and adsorption affinity are the fundamental aspects of study for assessing the feasibility of an adsorption process for practical application. Usual experimental protocol to quantify them is to determine the adsorption equilibria and enthalpy of adsorption. However, theoretical interpretation is highly essential for sorbent surface modification and/or molecular design of adsorbents with high adsorption selectivity and capacity and also providing favourable adsorption isotherm. Furthermore, understanding the basic phenomenon of adsorption equilibrium and kinetics in relation to the sorbent surface chemistry is also very essential. Thermodynamic measurements as regard affinities and enthalpies of adsorption on all known adsorbents are seldom reported in the literature. This exercise can enhance the capability to predict adsorption affinity/capacity of the secondary metabolites from certain bioactive compounds on particular adsorbents.

The major objective of this study is to investigate the equilibrium, kinetics and enthalpies of aqueous phase adsorption of certain pharmaceutically important bioactive compounds like *ginsenoside saponin* and *caffeine* with selected neutral polymeric resins like XAD-4, XAD-7, XAD-16 etc. for suggesting appropriate isotherm and kinetic model. The objective is also aimed to develop a knowledge base for design of suitable adsorbents with high selectivity and capacity for the
selected *alkaloids* or *saponins* through experimental studies and establish the feasibility of the adsorptive separation process in fixed bed columns to suggest appropriate configuration for design of commercial process through modeling and simulation exercises.

In order to achieve these major objectives, experiments were carried out with four specific objectives which have been formed considering needs to understand theoretical aspects involved and to relate the study to industrial processing applications through mathematical modeling. The specific objectives are:

1. Study adsorption parameters, which quantitatively describe the adsorption kinetics and equilibrium under various batch experimental conditions.
2. Study thermodynamic parameters in the adsorption process.
3. Investigate various kinetic, batch equilibrium and diffusion models and compare with experimental data.
4. Study column dynamics for the adsorption process under fixed bed conditions and simulate the data for commercial applications.

Background and reasons, which led to formation of the specific objectives, are described in the introduction of the individual chapters.
1.2. Organization of the thesis

This thesis consists of seven chapters. Following the introduction, objective and scope of study, chapter-1 includes description of the adsorption process, taking into account of related scientific concepts and an overview of various general theoretical frameworks which were used for the present study in this thesis. This chapter also covers properties of ginsenoside and caffeine, the two pharmaceutically important water soluble plant product that were used for carrying out the present research work.

Chapter-2 focuses on determining adsorption equilibrium. Adsorption isotherms were interpreted from various isotherm models and their parameters were evaluated and compared. These parameters are specific to the compounds studied and interactive polymeric adsorbents used under the experimental conditions employed.

Chapter-3 deals with adsorption kinetics. The adsorption kinetics was analyzed using series of rate equations like first and second-order rate kinetic equations, and intra-particle diffusion and diffusivity models for the adsorption processes under study. The adsorption performances were investigated thermodynamically under batch equilibrium conditions at different operating temperatures and are discussed in chapter-4.
The column dynamic studies under fixed bed conditions were studied and elaborated with numerical computations and modeling of axial and radial equations in chapter-5.

Chapter-6 provides the acronyms for symbols and abbreviations including the lists of tables and figures. The study concludes with chapter-7. It presents the recommendations for future work in respect of the findings of this research work. The literature surveys for each of the sub-topics are presented at the introduction of each chapter including the list of references at the end of the respective chapters. The appendix includes MATLAB codes and scripts for the numerical solution to the model equations with solution data and reprints of the publications made.

### 1.3 Adsorption – a brief description

The use of solids for removing substances from either gaseous or liquid solutions has been widely used since biblical times. This process, known as adsorption, involves nothing more than the preferential partitioning of substances from the gaseous or liquid phase onto the surface of a solid substrate. From the early days of using bone char for decolorization of sugar solutions and other foods, to the later implementation of activated carbon for removing nerve gases from the battlefield, to today's thousands of applications, the adsorption phenomenon has become a useful tool for purification and separation. Compounds that contain chromogenic groups (atomic arrangements that vibrate at frequencies
in the visible spectrum) very often are strongly adsorbed on activated carbon. Decolorization can be wonderfully efficient by adsorption and with negligible loss of other materials. Adsorption phenomena are operative in most natural physical, biological, and chemical systems, and adsorption operations employing solids such as activated carbon and synthetic resins are used widely in industrial applications and for purification of waters and wastewaters. The most common industrial adsorbents are activated carbon, silica gel, and alumina, because they present enormous surface areas per unit weight. Activated carbon is produced by roasting organic material to decompose it to granules of carbon - coconut shell, wood, and bone are common sources. Silica gel is a matrix of hydrated silicon dioxide. Alumina is mined or precipitated aluminum oxide and hydroxide. Although activated carbon is a magnificent material for adsorption, its black color persists and adds a grey tinge if even trace amounts are left after treatment; however filter materials with fine pores remove carbon quite well.

A surface already heavily contaminated by adsorbates is not likely to have much capacity for additional binding. Freshly prepared activated carbon has a clean surface. Charcoal made from roasting wood differs from activated carbon in that its surface is contaminated by other products, but further heating will drive off these compounds to produce a surface with high adsorptive capacity. Although the carbon atoms and linked carbons are most important for adsorption, the mineral structure contributes to shape and to mechanical strength. Spent activated carbon is regenerated by roasting, but the thermal expansion and contraction eventually disintegrate the structure so some carbon is lost or oxidized.
The process of adsorption involves separation of a substance from one phase accompanied by its accumulation or concentration at the surface of another. The adsorbing phase is the adsorbent, and the material concentrated or adsorbed at the surface of that phase is the adsorbate. Adsorption is thus different from absorption, a process in which material transferred from one phase to another (e.g. liquid) interpenetrates the second phase to form a ‘solution’. The term sorption is a general expression encompassing both processes.

Physical adsorption is caused mainly by Vander Waals forces and electrostatic forces between adsorbate molecules and the atoms which compose the adsorbent surface. Thus adsorbents are characterized first by surface properties such as surface area and polarity. A large specific surface area is preferable for providing large adsorption capacity, but the creation of a large internal surface area in a limited volume inevitably gives rise to large numbers of small sized pores between adsorption surfaces. The size of the micropores determines the accessibility of adsorbate molecules to the internal adsorption surface, so the pore size distribution of micropores is another important property for characterizing adsorptivity of adsorbents. Especially materials such as zeolite and carbon molecular sieves can be specifically engineered with precise pore size distributions and hence tuned for a particular separation. Surface polarity corresponds to affinity with polar substances such as water or alcohols. Polar adsorbents are thus called ‘hydrophillic’ and aluminosilicates such as zeolites, porous alumina, silica gel or silica-alumina are examples of adsorbents of this type. On the other hand, nonpolar adsorbents are generally ‘hydrophobic’.
Carbonaceous adsorbents, polymeric adsorbents and silicalites are typical nonpolar adsorbents. These adsorbents have more affinity with oil or hydrocarbons than water and are by and large used as separating agents to express the difference between molecules in a mixture. Polymeric adsorbents are usually microporous with high specific surface material (200 - 1000 m²/g). Some of the most commonly used adsorbents are shown in Table 1-1:
<table>
<thead>
<tr>
<th>Adsorbent</th>
<th>Basic Characteristics</th>
<th>Common use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alumina</td>
<td>Crystalline, Metastable</td>
<td>Hydrocarbon drying applications</td>
</tr>
<tr>
<td>Silica Gel</td>
<td>Amorphous, Beads</td>
<td>Drying of air, natural gas</td>
</tr>
<tr>
<td>Zeolite molecular sieves</td>
<td>Crystalline aluminosilicates</td>
<td>Gas &amp; liquid separations, drying</td>
</tr>
<tr>
<td>Mordenite, other natural zeolites</td>
<td>Repeating pore network</td>
<td>Drying of process air, CO₂, CO, natural gas</td>
</tr>
<tr>
<td>Silicalites or ZSMx</td>
<td>Hydrophobic carbon like, non polar</td>
<td>Application in water purification</td>
</tr>
<tr>
<td>Activated carbon</td>
<td>Microcrystallites with a graphite lattice</td>
<td>Gas &amp; liquid separations, decolorization</td>
</tr>
<tr>
<td>Carbon molecular sieves</td>
<td>Narrow pore distribution, large surface area</td>
<td>Gas separation</td>
</tr>
<tr>
<td>Impregnated carbons</td>
<td>Modified activated carbon</td>
<td>Cu-chlorides -- CO₂, H₂S, thiols separation</td>
</tr>
<tr>
<td>Polymeric resins</td>
<td>Multi-functional binding selectivities</td>
<td>Biological, ions, large molecules</td>
</tr>
<tr>
<td>Clays</td>
<td>Natural and pillared clays</td>
<td>Hydrocarbon</td>
</tr>
</tbody>
</table>

Table 1-1: Common adsorbents and their basic characteristics and use
1.4 Polymeric Adsorbents

Polymeric adsorbents are used for bulk separation and purification in food, pharmaceuticals and chemical industries. They are attractive due to their favourable elution and regeneration characteristics in spite of their lower adsorption capacity than the ion exchange resins and activated carbons. Adsorptions by neutral polymeric resins have many advantages like low toxicity, effective separation from very dilute aqueous solutions and special selectivity. Extensive studies have been made on the adsorption of various bio-molecules on neutral polymeric resins\textsuperscript{11-14}. The macroporous (macroreticular) polymeric resins have rigid three-dimensional structures and are most suitable to incorporate large amounts of extracts due to their high specific surface area, high mechanical strength, and rather low solvent swelling properties during the process of adsorption. In general, they have a specific surface area of 200–1000 m\textsuperscript{2}/g, a porosity of 0.4–0.6, an average pore diameter of 4–14 nm, and a pore volume of 0.6–1.8 cm\textsuperscript{3}/g. The Amberlite XAD resins from Rohm & Haas Co., are the widely-used commercial polymeric adsorbents. They are classified as:

(1) Aromatic, syrene-divinylbenzene copolymer (hydrophobic):
   XAD-2, XAD-4, etc.

(2) Aliphatic, methylacrylate (moderately hydrophilic):
   XAD-7, XAD-8, etc.

(3) Aromatic, divinylbenzene (hydrophobic):
   XAD-12, XAD-16, etc.
Other widely used such type of adsorbents are DOWEX Optipore from Dow Chemical Company, Lewatit from Bayer and Hypersol-Macronet from Purolite. Polymeric resins are typically based on cross-linked polymers having polystyrene, phenolformaldehyde, or acrylate matrices\(^\text{15}\) (Figure 1-1). Most commercial macroporous polymeric sorbents are based on polystyrene-divinylbenzene copolymers\(^\text{16}\) in which the divinylbenzene serves as a cross-linking agent that makes the styrene insoluble and confers physical strength to the resin\(^\text{17}\). Although they can be based on the same matrices, polymeric resins differ from traditional ion-exchange resins in their lack of ionic functional groups.

![Fig.1-1: Various matrices used for polymeric resins](image)

The present work reports experimental data on the batch equilibrium adsorption of the selected biomolecules caffeine and ginsenoside from aqueous solutions at various concentrations and temperature on to various Amberlite neutral resins of different properties. The polyaromatic adsorbents used in the study are Amberlite XAD-4, XAD-7, XAD-16 and XAD-1180. The physical properties and characteristics of the resins are reported in Table 1-2.
<table>
<thead>
<tr>
<th>Properties</th>
<th>XAD-4</th>
<th>XAD-7</th>
<th>XAD-16</th>
<th>XAD-1180</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical nature</td>
<td>Polystyrene</td>
<td>acrylic ester</td>
<td>Polystyrene</td>
<td>Polystyrene</td>
</tr>
<tr>
<td></td>
<td>divinyl benzene</td>
<td></td>
<td>divinyl benzene</td>
<td>divinyl benzene</td>
</tr>
<tr>
<td>Polarity</td>
<td>Non-polar</td>
<td>Weakly polar</td>
<td>Non-polar</td>
<td>Non-polar</td>
</tr>
<tr>
<td>Porosity</td>
<td>0.51</td>
<td>0.55</td>
<td>0.55</td>
<td>0.6</td>
</tr>
<tr>
<td>Pore volume, cm$^3$/g$^{-1}$</td>
<td>0.974</td>
<td>1.14</td>
<td>1.404</td>
<td>1.68</td>
</tr>
<tr>
<td>Particle size, mm</td>
<td>0.49-0.69</td>
<td>0.25-0.84</td>
<td>0.30-1.20</td>
<td>0.35-0.60</td>
</tr>
<tr>
<td>Mean pore dia, Å</td>
<td>50</td>
<td>80</td>
<td>100</td>
<td>140</td>
</tr>
<tr>
<td>Density, g ml$^{-1}$</td>
<td>1.035</td>
<td>1.07</td>
<td>1.02</td>
<td>1.04</td>
</tr>
<tr>
<td>Surface area, m$^2$/g$^{-1}$</td>
<td>750</td>
<td>450</td>
<td>825</td>
<td>600</td>
</tr>
</tbody>
</table>

Table 1-2: Properties of Amberlite polymeric adsorbents
1.5 Caffeine

Caffeine has wide range of significant uses in food products and drugs. Caffeine (1, 3, 7-trimethylxanthine), structure shown in Fig.1-2 is the major alkaloid in many herbs including tea, coffee, cocoa etc. Excess dose of caffeine in caffeinated drinks increases the risk of health hazards like cerebral, gastrointestinal, cardiac and psychological disorders. Decaffeination or recovery of caffeine has been an important step in the caffeine related industrial productions. Various technologies have been used for the extraction of caffeine. Traditional methods include organic solvent extraction, water extraction, supercritical CO\(_2\) extraction and use of varieties of isolation methods like charcoal adsorption, reverse osmosis, re-crystallization, distillation etc. Caffeine extraction through use of different organic solvents like benzene, chloroform, trichloroethylene, ethyl acetate and dichloromethane though being used over the years, have limitations for reasons of environmental considerations like toxicity and chemical residues. More effectively the cost and flavor factors also play an important role. These processes have therefore been gradually replaced by water extraction and other methods. Supercritical CO\(_2\) is an excellent nonpolar solvent for caffeine and is safer than the organic solvents that are used for caffeine extraction. But the process is not cost effective. Conventional separation processes use the advantage of one of the physical and chemical properties like electrostatic charge, hydrophobicity, molecular size, solubility etc. In short all these conventional extraction methods used for decaffeination or extraction of caffeine.
have their own limitations from environmental considerations, cost effectiveness, solvating ability and poor separation for complex surface chemistry of adsorbents.

![Caffeine (1,3,7-trimethylxanthine)](image)

Fig.1-2: Caffeine (1,3,7-trimethylxanthine)

### 1.6 Ginsenoside

Panax Ginseng, a member of Araliaceae family, is a traditionally famous oriental medicinal plant. Ginsenoside saponins from Ginseng are the most important active components in Ginseng roots and are attributed with cardio-protective, immunomodulatory, anti-fatigue and hepato-protective physiological and pharmacological properties. The major groups of Ginsenoside Saponins are the Rb and Rg groups derived from the Protopanaxadiol and Protopanaxatriol structures, respectively. The Molecular structure of the Protopanaxadiol and Protopanaxatriol are as follows:

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1-17
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Protopanaxadiol

Protopanaxatriol

Fig. 1-3: Ginsenoside
It has been claimed that *ginseng* has many beneficial bioactive effects on human health. It is estimated that the current world sales of various *ginseng* raw materials have reached about one billion US dollars per annum\(^2\). The medicinal value of *ginseng* has also been of vast interest worldwide and extensive effort is being put into investigating its pharmacological effects. Purified *ginseng* *saponins* are very expensive due to its low yield in the *ginseng* roots. Plant cell cultures are an alternative source to whole plant for the efficient production of the valuable secondary metabolites. Although *ginseng* cell culture has so far found limited commercial application, it is an active current area of research and development around the world and possesses great potential for mass industrialization.

A highly efficient extraction and purification technique for isolation of the bioactive molecules like \(Rb\) & \(Rg\) *ginsenosides* from the cultured cell mass is therefore very important to reduce its cost and to have wide pharmaceutical applications. The traditional methods of solvent extraction of plant materials are mostly based on the choice of solvents and use of heat and/or mixing to increase the solubility of and the rate of mass transfer.

Different extraction methods including conventional organic solvent, heat reflux, ultrasound assisted and microwave assisted extraction methods were attempted for efficient extraction of *ginseng* *saponins* from the cell mass of *ginseng* root obtained in bioreactor cultures. Ultrasound assisted extraction enhances both solvent penetration into the plant materials and the release of
intracellular product by disruption of the cell walls mainly due to the mechanical effects of the acoustic cavitations\textsuperscript{2}.

Extraction by micro-wave assisted technique which combines microwave with the traditional method of solvent extraction\textsuperscript{6} is another method in practice. Microwave assisted extraction depends on the dielectric susceptibility of both solvent and the cell matrix. Microwave energy causes motion by migration of ions and rotation of dipoles and therefore depends on the presence of polar molecules or ionic species. This is relatively new technique of secondary metabolite extraction from plant materials and yet to find commercial applications as far as the \textit{ginsenoside saponin} extraction is concerned.

All these extraction methods have their merits and demerits from the viewpoint of applicability and large scale economic extraction. There is therefore wide scope of making proper scientific study for upgrading the \textit{ginsenoside saponin} extraction process and technology for efficient and economic use.

Adsorption chromatography is a recommended process for extraction and purification of synthetic compounds and the adsorbents known from literature are activated carbon, molecular sieves and non-ionic/ ion exchange resins. Though much work has been done to improve the separation of various compounds by analytical liquid chromatography, systematic investigations on \textit{ginsenoside saponin} extraction have not been made. There is therefore limited knowledge on the details of the adsorption process for \textit{ginsenoside saponins}.
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