CHAPTER 2
LITERATURE REVIEW

2.1 Introduction of Membrane Separation

In biotechnology, membranes have been used for separation processes for some time (McGregor, 1986). Membrane processes are technically simple, have high efficiency, and for solid membranes, the components to be separated are not altered chemically or thermally (Marr and Kopp, 1982).

Liquid membrane technology or transport of solute across thin film of organic solvent is the process in which an extraction process occurs by means of a selective liquid separating phase (the liquid membrane). The transport of a dissolved solute through this liquid membrane gives rise to the process called liquid membrane extraction (Marr and Kopp, 1982). This technology which has not so far found much chemical application in the bioprocessing industries.

Research in the field of liquid membrane extraction has mainly focused on application in the recovery of metal ions. Where liquid membranes have been used in biochemicals, there have been no models developed to describe and predict the extraction kinetics. A problem occurred with emulsion liquid membranes is swelling, water transport across the organic film. Liquid membrane can be classified by configuration of membrane phase which is known as **a supported liquid membrane (SLM)** and **an emulsion liquid membrane (ELM).** Supported liquid membranes have membrane areas of 100-200 m^2/m^3 of equipment volume and emulsion liquid membranes have surface areas of 1000 to 3000 m^2/m^3 (Marr and Kopp, 1982).

Since their discovery by N. Li in 1968, emulsion liquid membranes have been considered as effective equipment for a wide variety of separation such as the removal of zinc from wastewater in the viscous fiber industry (Draxler, Furt and Marr, 1988), the recovery of nickel from electroplating solutions (Marr, Lackner and Bart, 1989), biochemical processing application including the separation of amino acids, antibiotics and phospholipids, and the recovery of organic acid both from broth fermentation (Thien, Hatton and Wang, 1986), biomedical applications including blood oxygenation (Li and Asher, 1973); alkaloid extraction from plants with liquid membranes (Tang, Ma and Liu, 1990); emulsion liquid membrane extraction of Phenylalanine and Tryptophan (Noppaporn Panich, 1994); and extraction of L-lysin from aqueous solution by emulsion liquid membrane process (Apirak Suetrong, 1995).

2.2 Application of Emulsion Liquid Membranes

2.2.1 Alkaloid Extraction from Plants with Liquid Membranes

Tang, Ma and Lui, (1989) conducted extraction of alkaloid from Rhizoma Coptidis by means of liquid membrane technology. The plants were selected by qualitative examination. The external (feed) phase was aqueous berberine solution from Rhizoma Coptidis at various acidity. Using kerosene as membrane material (organic solution) and Span-80 at various volume percent from 1% to 4% (v/v) as emulsifier. The internal (stripping) phase was hydrochloric acid at various concentrations from 0.1 to 0.4 M. It was found in the experiments that the amount of berberine extracted increases with the increase in pH value of the external phase. At higher pH value, the opposite occurs due to the increased breakage of the liquid membrane at that pH. Any increase of Span-80 in the membrane from the basis of

1% will result in the lowering of the amount of berberine extracted. The increased Span-80 causes a higher membrane viscosity, and thus a lower berberine flux. and the optimum hydrochloric acid in membrane was 0.3 M. Any deviation from this leads to a drop of berberine extraction when other conditions are held equal.

2.2.2 Emulsion Liquid Membrane Extraction of Phenylalanine and Tryptophan

Nappaporn Panich (1994) worked with extraction of two essential amino acids; L-phenylalanine and L-tryptophan, by emulsion liquid membrane from dilute solution. This experiment studied the equilibrium extraction of mixture and batch extraction of aqueous phenylalanine solution, aqueous tryptophan solution and mixture of both aqueous solutions. The external phases were both amino acids. The membrane phase was cation carrier D2EHPA and surfactant Span-80 dissolved in n-Dodecane. The internal phase was 1N hydrochloric acid. It was found that tryptophan had higher flux than phenylalanine. The extraction rate at pH 5 and pH 3 was higher than pH 2. In the extraction of mixture solution of tryptophan and phenylalanine, tryptophan did not have significant effect on the transport rate of phenylalanine.

2.2.3 Kinetic Studies on the Emulsion Liquid Membrane Extraction of Lactic Acid

Julian Brajendra Chaudhuri (1990) conducted batch extraction of lactic acid (0.09-1.0 M) from model and real fermentation broths which was carried out in a stirred vassal (300 cm^3). The liquid membrane emulsion contained a stabilizing

surfactant, Span-80 [sobitan monooleate (1-10% v/v)], a carrier species (trioctylamine) as Alamine 336 (0-10% v/v) and a 70% n-heptane/30% paraffin mixture used as the organic diluent. Sodium carbonate solutions (0.28-1.9 M) were used as the stripping phase. The external (feed) and internal (stripping) phases solute concentrations were measured, and through mass and volume balance the phase volume and osmotic pressure variations were monitored. The experimental result showed that a shrinking core model was necessary to describe solute transport in the emulsion globules and swelling did not significantly affect the rate of solute depletion from the feed phase. The kinetic model was able to predict the effects of variations in parameters such as the carrier concentration, and the feed and stripping phase concentrations. For extraction selectivity, it was found that the glucose and sucrose, initially in the feed phase, accumulated in the stripping phase. Extraction of lactic acid from fermentation broth was poor in comparison to results obtained from the model systems, because of competition for the carrier by hydrochloric acid used to adjust the pH.

2.2.4 Extraction of L-Lysinees from Aqueous Solution by Emulsion Liquid Membrane Process

Apirak Suetrong (1995) studied batch extraction of aqueous L-lysine solution by emulsion liquid membrane process. The membrane phase consisted of the cation carrier D2EHPA and the surfactant Span-80 which dissolved in n-Dodecane. The internal aqueous phase was hydrochloric acid solution. The experimental conditions were varied for the determination of the optimum conditions. It was found that the optimum condition for the external phase was 1 mM of L-lysine at pH 5. The optimum conditions of the membrane phase were 5 % (v/v) Span-80 and 10% (v/v) D2EHPA dissolved in n-Dodecane. The optimum condition of the external phase was 1 M HCl.

The agitation speed that was good for extraction was 420 rpm. 50% of L-lysine from aqueous solution in the external phase was extracted within 5 minutes. At the final extraction, the concentration of L-lysine in the internal phase was doubled from the external phase.

2.3 Definition of Alkaloids

From ancient time man has utilized alkaloids as medicines, poisons and magical potions. Only recently has he gained precise knowledge about the chemical structures of many of these interesting compounds. The term alkaloid, or **"alkali-like"**, was first proposed by the pharmacist, as nitrogen-containing compounds of plant origin (William, 1983).

The precise definition of term **"alkaloid"** is somewhat difficult because there is no clear-cut boundary between alkaloids and naturally occurring complex amines. Typical alkaloids are derived from plant sources. It is usually applied with the basis of nitrogen-containing compounds involved in a heterocyclic ring manifested significant pharmacological activity. The nitrogen of alkaloids, taken in their broadest sense, may have a nitrogen atom which is primary, secondary, tertiary or quaternary and this factor affects the derivations of the alkaloid which can be prepared and the isolation procedures.

In the plant, alkaloids may exist in free state, as salts or as amines or alkaloid N-oxides. There are numerous groups of alkaloids isolated from the lower plants, animals, microorganism, flowering plants and marine products. In the higher plant system of Engler, there are 60 orders and 34 of these contain alkaloid-bearing species. The most important alkaloid-containing families are the Liliaceae, Amaryllidaceae, Compositae, Lauraceae, Ranunculaceae, Menispermaceae, Papaveraceae,

Leguminosae, Rutaceae, Longaniaceae, Apocynaceae, Solanaceae and Rubiaceae (William, 1983).

2.4 Introduction to Berberine

In Thailand, traditional medicine named **"Khamin Khruea"** refers to the woody climber with yellow wood. Khamin Khruea is classified as Menispermaceae family and distributed in the tropical rain forest regions of Asia including Thailand. There are many different species under the name Khamin Khruea but they have been used in the same therapeutic purpose in folkloric medicine.

Various parts of Khamin Khruea have been used in pharmaceutical utilization for a wide variety of diseases such as fever, colic, muscle pain, stomach pain and as antidiarrhoea and antifungal (Bhakuni, 1984). In folkloric medicine people favor to use stems and roots of Khamin Khruea than leaves because of the richness of their alkaloid contents.

In India, this plant known as "Dharhaidi" is used to prepare a yellow dye. It is also widely **used as a medicine**: aqueous or alcoholic extracts are used as a bitter tonic, a paste of pounded roots and stems is used to dress bruises and contusions. Darvi and ayurvedic drug are used against ulcers and affections of the eye.

The main alkaloid of Khamin Khruea is berberine that may exists in free state, (see the chemical structure in Figure 2.1) but for practical considerations it must be more restrictive with some exception. Berberine is in isoquinoline alkaloid group which had played an important part in the development of the chemical and biological science (Supranee Keawpradub, 1992).

Berberine containing plants are used in traditional medicine against illness affecting the digestive tract, e.g. gastric ulcers and against infections (oriental sore,

conjunctivitis). Local injections of berberine sulphate are also used to cure dermal leishmaniasis. The berberine alkaloids are reported to have moderate, slow and selective antimicrobial activity with a low toxicity. So far no allergic reaction or local side effect are known to occur after therapeutical application of berberine type alkaloids (Bhakuni, 1984).

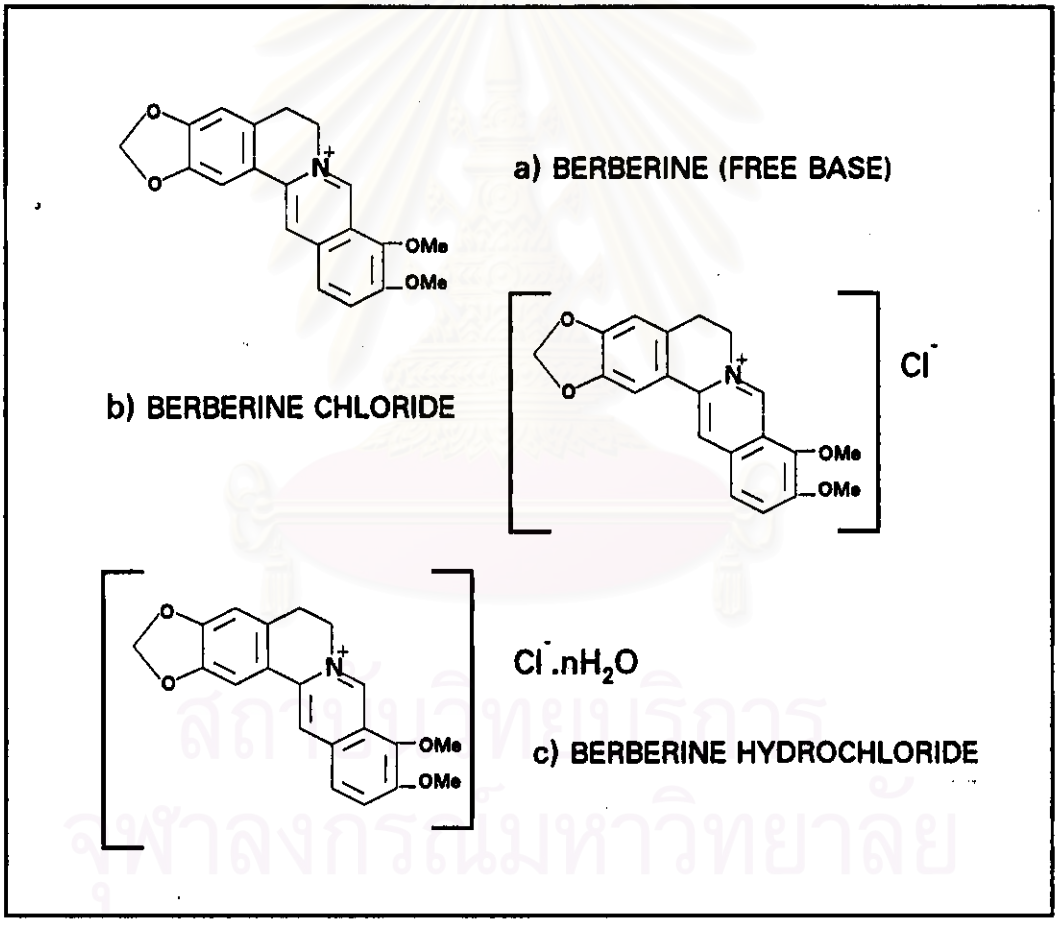

Figure 2.1 a) Chemical Structure of Berberine (Free Base)

b) Chemical Structure of Berberine Chloride

c) Chemical Structure of Berberine Hydrochloride

In salt forms of berberine, berberine chloride is a quaternary ammonium salt ($R_4N^+X^-$) which is yellow crystalline powder, odorless, bitter taste, soluble in hot water, slightly soluble in water or ethanol, very slightly soluble in chloroform and insoluble in ether. In the form of free base, it can be dissolved in organic materials such as chloroform, kerosene and ether (Pharmacopoeia of the People's Republic of China, 1992).