CHAPTER II

LITERATURE REVIEWS

Today the topic of microencapsulation is extensively studies inside major pharmaceutical companies and universities as well as research institutes. Microencapsulation is one of the intriguing fields of drug delivery systems. It requires knowledge of pure polymer sciences, emulsion technology, and in-depth understanding of drug and protein stabilization. Although scientists at the beginning of the 1970s were primarily concerned with the encapsulation of dyes to produce carbonless paper, scientists today have mastered the technology to such a level that cell as well as delicate protein and gene can be encapsulated (Mathiowitz, 1999).

1. Classification of Microsphere and Microcapsules

A microcapsule can be described as a system that contains a well-defined core and envelope: the core can be solid, liquid or gas, the envelope is made of a continuous porous or nonporous, polymeric phase. At present, there is no universally accepted size range that particles must have in order to classified as microcapsules. In general microcapsules are small particles, many workers classify capsules smaller than 1 μm as nanocapsules and capsules larger than 1,000 μm as macrocapsules. Commercial microcapsules typically have a diameter between 3-800 μm. Microcapsules can have a variety of structures; some have a spherical geometry with a continuous core region surrounded by a continuous shell, others have an irregular geometry and contain a number of small droplets or particle of core material as shown in figure 1a. Alternatively, a microsphere is a structure made of a continuous phase of one or more polymers in which particulate drug is dispersed (figure 1b). However, the difference between the two systems is the nature of the microsphere matrix, in which no well -defined wall or envelope exists. Different methods of encapsulation result in either a microcapsules or microsphere. For example, interfacial polymerization almost always produces microcapsules whereas solvent evaporation may result in microspheres or microcapsules depend on the amount of loading. In addition the shell of these

microcapsules or microspheres can be made flexible, brittle, or hard by means of selectivity the type of shell materials. For example, shell materials can be natural macromolecules such as gelatin, gum arabic, albumin, and sodium alginate or synthetic macromolecules such as polyvinyl alcohol, nylon, polyurethane, polyester, and epoxy. These materials are used singly or compounded in encapsulation process.

Wall materials

A variety of inorganic and organic materials can be used as wall materials, but polymeric substances are used most frequently. The wall material is selected appropriately depending on the physical properties of the core material. If the core is lipophilic, a hydrophilic polymer is used as the wall material. When aqueous solution is used as a core material, a water insoluble synthetic polymer is used as the wall material. Examples of commonly employed wall materials are as follows (Kondo, 1979):

Proteins i.e. collagen, gelatin, casein, polyamino acid, and albumin.

Vegetable gums i.e. ethylcellulose, nitrocellulose, cellulose acetate-phthalate and cellulose acetate-butylate-phthalate.

Condensation polymers i.e. nylon, polyurea, polycarbonate, silicone resins.

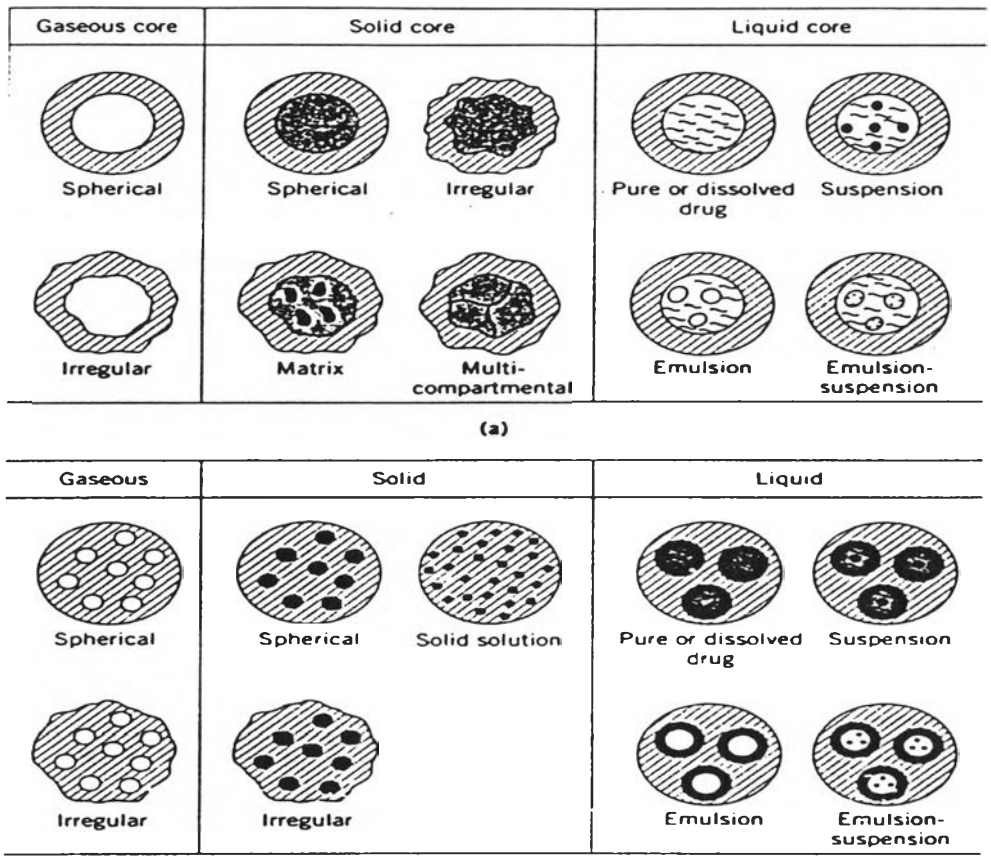
Copolymer i.e. maleic anhydride copolymers with ethylene or vinyl methyl ether.

Homopolymers i.e. polyethylene, polyvinyl alcohol, polyacrylamide.

Waxes i.e. paraffin, shellac, tristearin and beeswax.

Inorganic materials i.e. calcium sulfate, graphite silicate and clays.

Figure 1 Microcapsules (a) and microspheres (b) (Mathiowitz,1999).



2. Classification of Microencapsulation Techniques

Microencapsulation is a technology devoted to entrapping solids, liquids or gases inside of more polymeric coating (Becher, 1983). Two major classes of encapsulation methods have involved chemical and physical. The first class of encapsulation involves polymerization during the process of preparing the microcapsules, example of this class is well known by the name of interfacial polymerization. The second type involves the controlled precipitation of a polymeric solution wherein physical changes usually occur.

In table 1 the examples of different types of methods are demonstrated according to published literature. It is sometimes difficult to classify encapsulation methods because specific techniques can be hybrid of two or more methods or can use

different mechanism simultaneously. Also many names have changed throughout the years e.g. solvent evaporation has been called water drying or double emulsion and this can create confusion for readers.

Table 1 Illustration of microencapsulation methods

Process	Coating material	Suspended medium
Interfacial polymerization	Water-soluble and insoluble monomers	Aqueous/organic solvent
Complex coacervation	Water -soluble polyelectrolyte	Water
Coacervation	Hydrophilic or hydrophobic polymers	Aqueous/organic solvent
Thermal denaturation	Proteins	Organic solvent
Solvent evaporation	Hydrophilic or hydrophobic polymers	Organic or water
Solvent removal	Hydrophilic or hydrophobic polymers	Organic solvent
Phase separation	Hydrophilic or hydrophobic polymers	Aqueous /organic
Hotmelt	Hydrophilic or hydrophobic polymers	Aqueous/organic solvent
Spray- drying	Hydrophilic or hydrophobic polymers	Air, nitrogen

These microencapsulation procedures are summarized briefly as follows:

2.1 Interfacial Polymerization

This technique was initially developed by Chang in Canada in the 1960s, Kondo and co- workers in Japan had also done extensive work on these system. Interfacial polymerization is one of the microencapsulation techniques using two reactive monomers, which are dissolved in immiscible solvents. One monomer is dissolved in aqueous phase and form emulsion with the solvent containing surfactant. The monomer diffuses to the oil-water interface where they react to form a polymeric membrane. The interfacial reaction occurs rapidly therefore, it is considered as a rapid of preparing microcapsules. The basic feature of this method is the formation of water in oil as

illustrated in figure 2. In this process, various combinations of monomers can be used to obtain a range of polymer membrane. Some possibilities are illustrated in figure 3 such as sebacoyl chloride and hexamethylene diamine(1,6-hexanediamine) to form the polyamide nylon 6,10. Polyurea and polyurethane can also be produced by this technique (Whateley, 1996).

This method is suitable for encapsulating liquid rather than solid since reactant can enter more easily into polymerization zone from a liquid than a solid phase. Chang et al. prepared poly (hexamethylene sebacamide) microcapsules by this reaction. The polymer was dispersed in carbonated buffer pH 9.8; HCl was formed during the polymerization. The buffer served to neutralize HCl step. Sorbitian trioleate (Span85®) was used as an emulsifying agent and sebacoyl dichloride as cross-linking agent. Microcapsules were obtained (Chang, Macintosh and Mason, 1966). Nevertheless, they tend to be rather fragile, difficult to separate, wash and handle. More robust microcapsules can be prepared using terephthalic (p-phthalic) acid dichloride with the diamine. Piperazine has frequently been used with terephthaloyl chloride to give a robust poly (terephthaloyl-piperazine) membrane (Whateley, 1996).

Factors Regarding the Interfacial Polymerization

1. **Concentration Ratio of Reactants**

The concentration ratio of the reactants can be important when high molecular weight polymer is desired. In generally, one reactant is used in excess. Microcapsules encapsulated pesticides prepared by diamine can be used in excess when its partition coefficient is unfavorable for its transfer to the organic solution as is established when lysine is used (Whateley, 1996).

2. **Stirring Rate**

Stirring rate is the critical variable and is found to affect the molecular weight of various types of polymers. The stirring produced by magnetic stirrer, as would be commonly available in a laboratory, can produce microcapsules down to 10-20 μm

while high-speed impeller in a special reaction vessel gave small microcapsules (diameter about 1 μm) (Wakamatsu, Koishi and Kondo, 1974).

3. Transfer Rate of Salts

An important consideration is the elimination of HCl formed in the polycondensation reaction. This product is removed by the formation of hydrochloride salt of the diamine which being poorly soluble in the organic phase. Diffuse to the aqueous phase when HCl is neutralized, salt of diamine are able to react with diacid chloride and therefore if buffer is not included in the aqueous phase, the diamine itself will function as an acid receptor. In most cases, the transfer of hydrochloride salts will be faster than the transfer rate in the opposite direction of diamine to the reaction site (Whateley, 1996).

4. Surfactant Concentration

The addition of surfactant in microencapsulation procedures is important for the formation of the emulsion. Surfactant also plays an important role in the transfer of the diamine to the organic phase. In general increasing the concentration of surfactant results in an increase in the amount of diamine that is transfered to the organic phase (Koishi, Fukuhara and Kondo, 1969). The main requirement of any surfactant is that it does not react with diacid chlorides and it does not have impurities, which would interfere with the polymerization reaction. The choice of surfactant used in studies where aqueous solutions are encapsulated in microcapsules has been limited mainly to sorbitan trioleate, which has a particularly low HLB (hydrophilic-lipophile balance) value (i.e., 1.8).

5. Temperature

Condensation polymerization reaction is usually carried out at room temperature. No advantage is found in heating the reaction and many preparations are improved by controlling the temperature rise. Some reactions proceed well at 4 $^{\circ}$C for their stability. (Rambourg, Levy and Levy, 1982).

6. Solvent

Chloroform-cyclohexane mixtures in the rations of 1:4 and 1:3 have been widely used. Polymer-solvent interaction is expected from chloroform system, whereas cyclohexane produces thin film (Morgan, 1965). Good solvent tends to produce high molecular weight polymer when compared with nonsolvents. For pharmaceutical applications, organic solvents have been chosen from solvents with low toxicity and avoid toxic solvents.

7. Diamine Partition Coefficient and Interfacial Transfer Rates

The partition coefficient of the diamine is easily measured at equilibrium and served as a guide to exclude monomers, which would be unsuitable for this type of reaction. The rate of transfer should exceed the rate of removal of diamine by polymerization.

Partition coefficient is defined as the ratio of the concentration of diamine in the aqueous phase to that in the organic phase. The values can be used to estimate the relative tendency of the diamine to transfer into the organic phase under polymerization conditions. Polymer formation is favorably affected by a large transfer of the diamine. For example, hexamethylene diamine in organic solvent, partition coefficient (K) of hexamethylene diamine in cyclohexane is 182 while K in xylene is 50 (Kondo, 1978).

The factors effecting microencapsulations were investigated in previous studies, for example, pH of the aqueous phase during interfacial polymerization, duration of the polymerization, surfactant concentration and stirring rate. Also various proteins and bifunctional acylating agent were investigated. The obtained microcapsules were spherical, with their sizes ranging from 50-150 μm. Lowering the pH of the aqueous phase resulted in loss of activity, An increase in pH value in aqueous solution was necessary for membrane formation. Optimal duration of polymerization step was 3-5 min., shorter time gave unstable capsules while longer time yielded a thick membrane. With no surfactant used, no microcapsules were formed. Increasing the concentration of the surfactant solution to1-2% have little effect on the average size (87-73 μm) but resulted in more homogeneous repartition of the diameters. When the stirring rate was

raised from 450 to 1200 rpm, the size of the microcapsules decreased regularly, while the distribution of their diameters became more and more homogeneous.

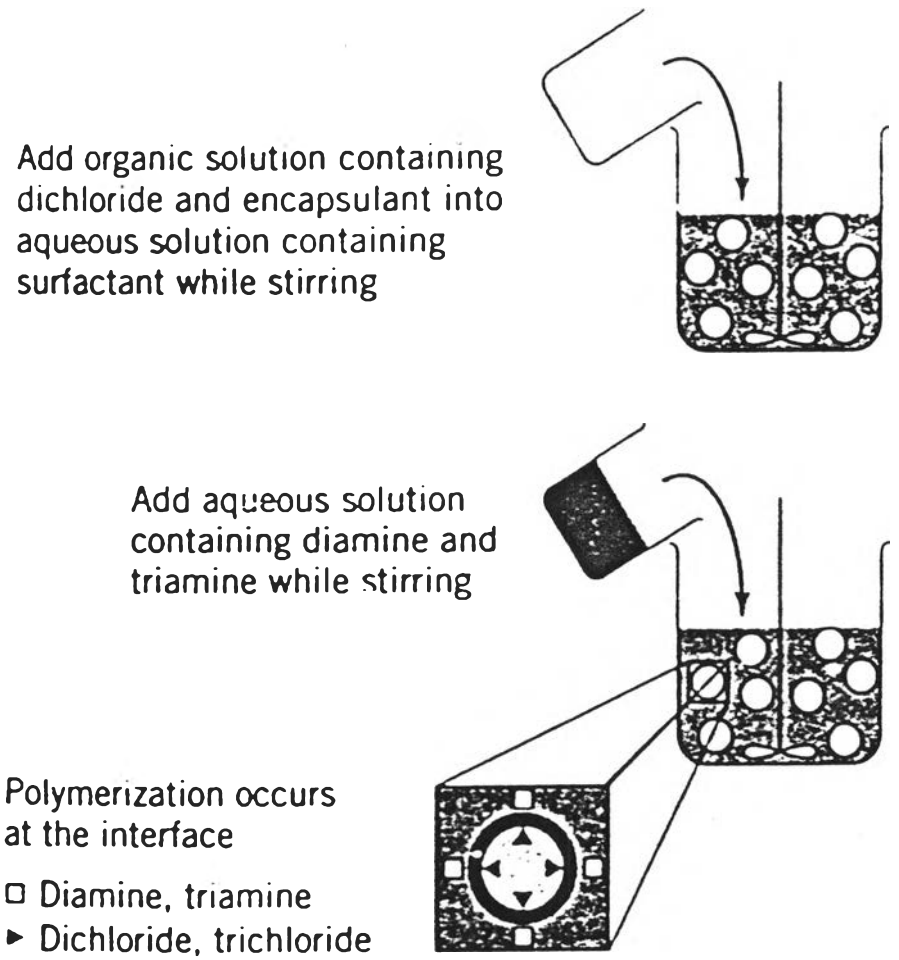


Figure 2 Microencapsulation by interfacial polymerization (Mathiowitz, 1999).

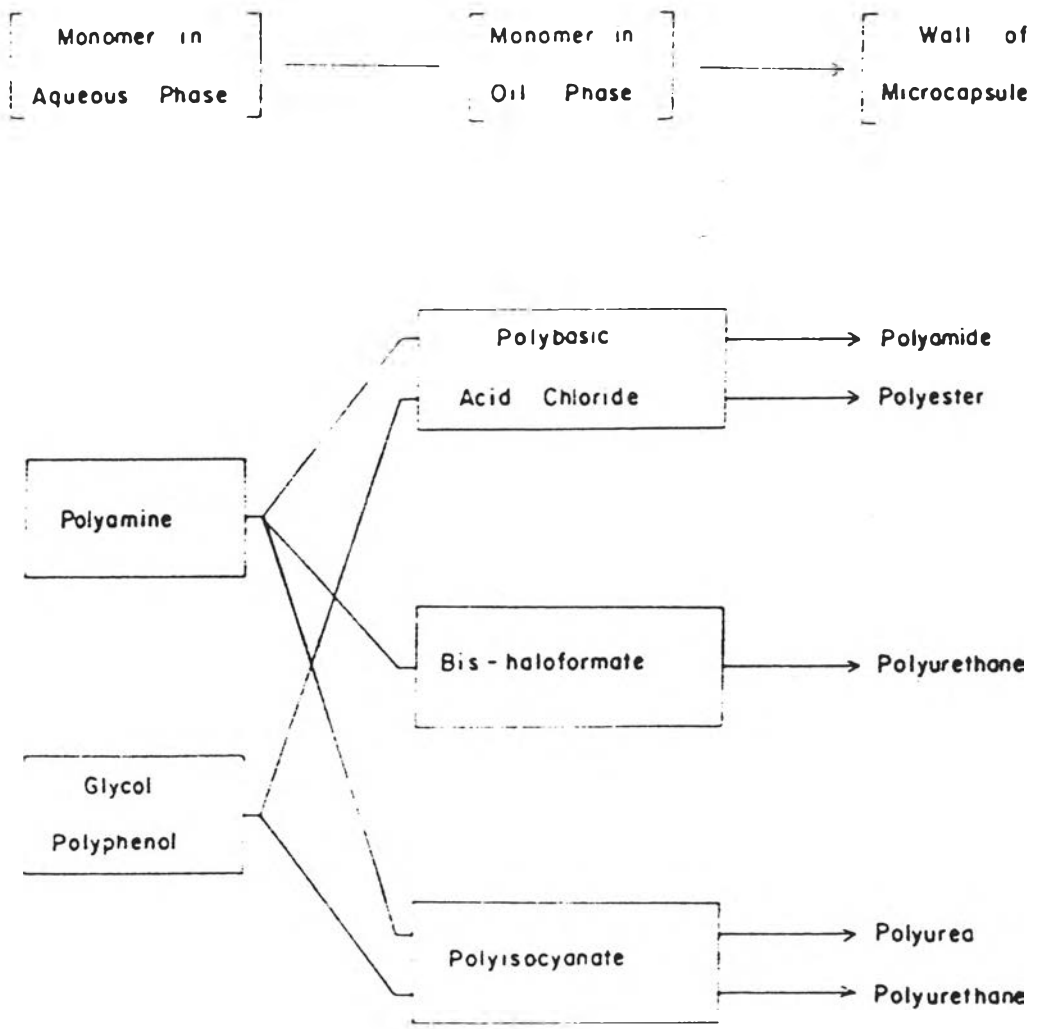


Figure 3 Examples of interfacial polymerization (Kondo, 1979).

2.2 Complex Coacervation

It was the process used to make microcapsules in the first successful encapsulated product, carbonless copy paper. The process uses the interaction of two oppositely charged polyelectrolytes in water to form polymer-rich coating solution called a coacervate. This solution engulfs the liquid or solid being encapsulated, thereby forming an embryo microcapsule. Cooling the system causes the coacervate to gel via network formation. Gelatin is a primary component of most complex coacervation systems. A schematic diagram of this encapsulation process of cationically charged gelatin by a negatively charged of gum arabic is illustrated in figure 4.

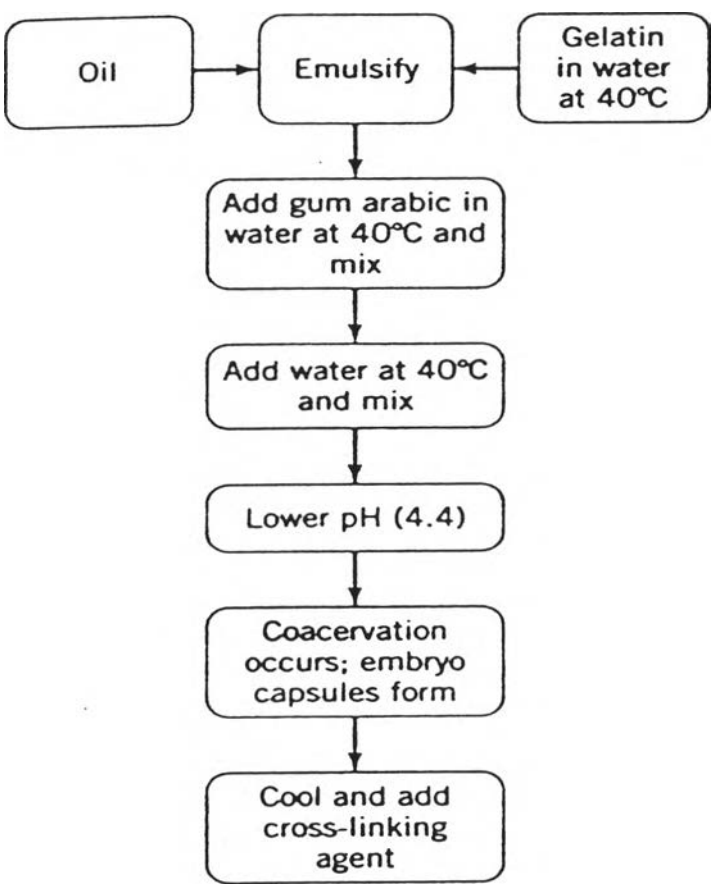


Figure 4 Microencapsulation procedure of complex coacervation by interaction of two oppositely charged polyelectrolytes (Mathiowitz, 1999).

2.3 Coacervation

Coacervation is one of the oldest and common microencapsulation techniques in current use. The term coacervation is used to describe the phenomenon of salting out or phase seperation of lyophilic colloids into liquid droplet rather than into solid aggregates (Deasy, 1984;Bakan and Doshi, 1991). The colloid phenomenon of coacervation was first described by Bungenberg de Jong and Kruyt in 1930 (from the Latin acervus , meaning aggregation or heap and the prefix Co to signify the prior union of the colloidal particles). Microencapsulation is achieved by allowing the colloidal- rich phase, the coacervate, to deposit around the core material, followed by gelation and insolubilization of the deposited coacervate. Simple coacervation can be brought about in any aqueous polymer solution provided that temperature, pH, solvent or salts are properly chosen. A typical example is the addition of sodium sulfate to oil in water emulsion, which is formed by using gelatin (Kondo, 1978).

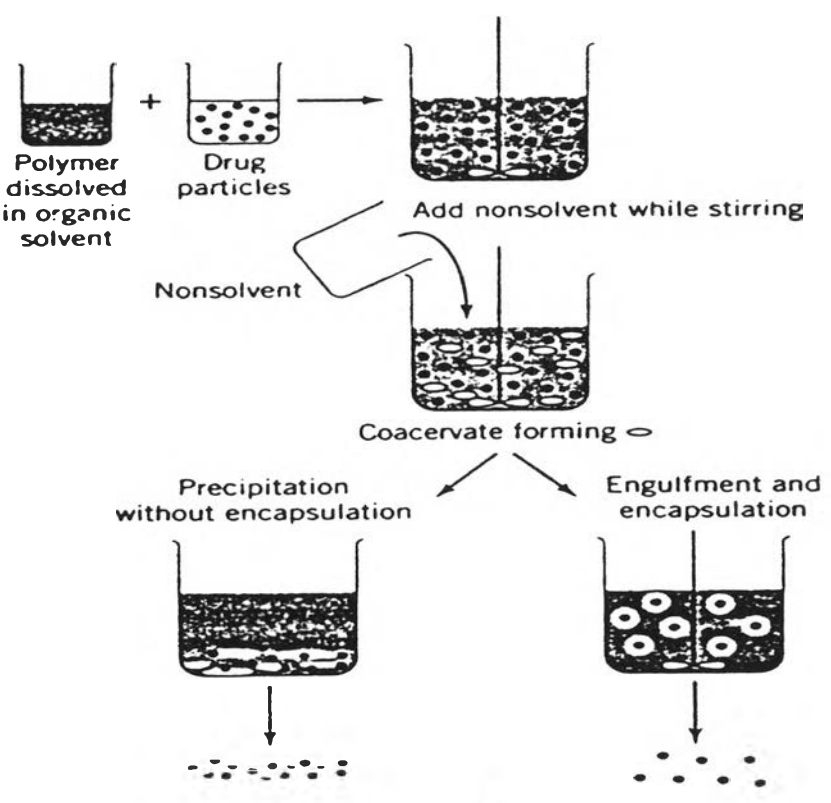


Figure 5 Microencapsulation procedure of coacervation (Mathiowitz, 1999).

2.4 Thermal Denaturation

Water-soluble proteins are sensitive to heat and can denature when heated. The denaturation process causes the protein chain to unfold and become chemically cross-linked. This insolubilizes the protein and creates a convenient method of forming protein microspheres. Proteins such as albumin and egg albumin in aqueous solvent (20%w/w) are used and drugs such as hydrochlorothiazide, sodium salicylate and enzyme hyaluronidases are prepared by this method (Modena et al., 1998). Figure 6 is a schematic diagram of thermal denaturation.

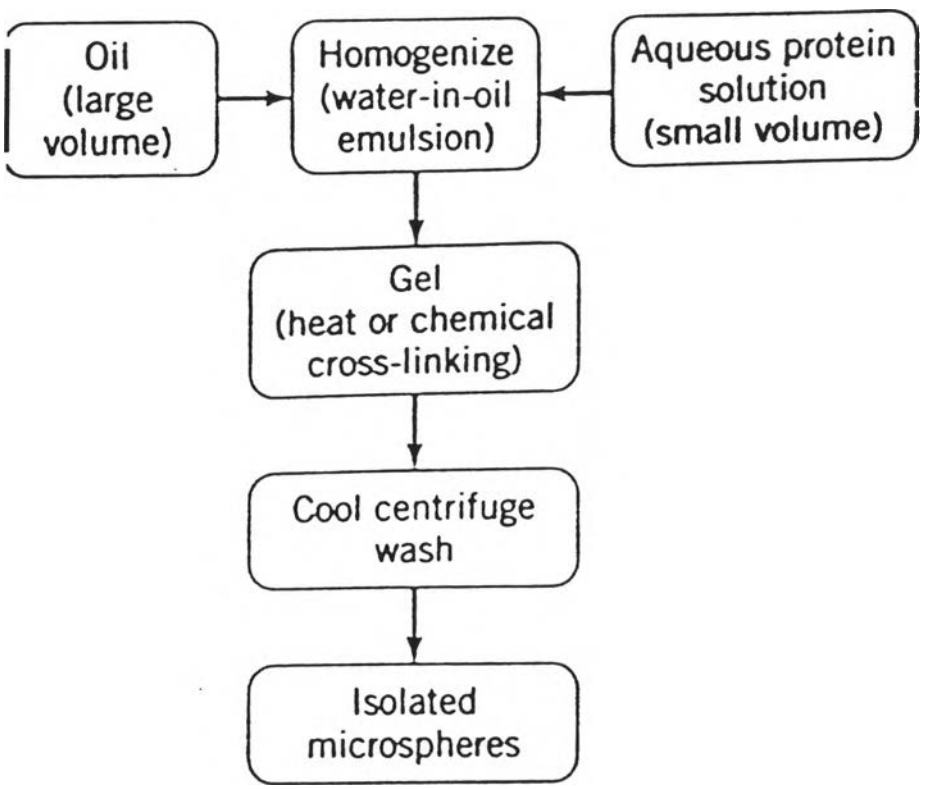


Figure 6 Microencapsulation by thermal denaturation (Mathiowitz, 1999).

2.5 Solvent Evaporation

One of the oldest and most widely methods of microcapsules preparation is solvent evaporation technique. The solvent evaporation encapsulation process is a way of precipitating small polymer particles from an oil-in water emulsion. The polymer is dissolved in a volatile organic solvent that is immiscible with water. Methylene chloride is a preferred solvent because of its high volatility (boiling point 41° C) and its capacity for dissolving a broad range of polymers. The polymer is dissolved in the organic solvent; the drug to be capsulate is added to this solution. The drug agent may be a solid or a liquid. The added drug may completely dissolve in polymer solution or insoluble and simply form dispersion, or suspension-emulsion. The drug /polymer/solvent mixture is emulsified in water to form an oil in water emulsion. A surfactant is normally dissolved in the water phase before the oil in water emulsion is formed. The system is stirred at a constant rate and the solvent evaporates. Once the solvent evaporate appears to be complete, the microcapsules are separated from the suspending medium by filtration, washed and dried. Ascorbic acid microcapsules were prepared by this method with ethylcellulose as polymer and acetone as polymer solvent (Vanichtanunkul, 1997). A diagram of solvent evaporation technique is illustrated in figure 7.

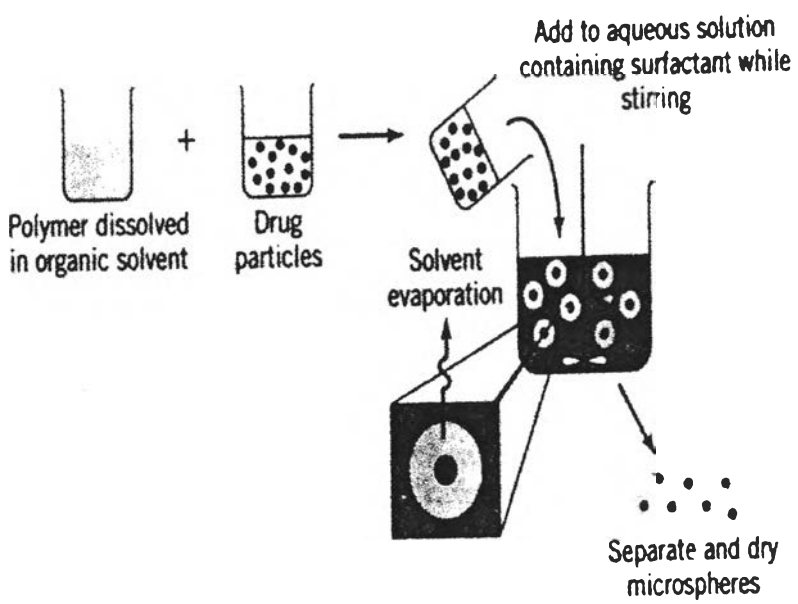


Figure 7 Microencapsulation by solvent evaporation technique (Mathiowitz, 1999).

2.6 Solvent Removal

This method is a modification of organic phase precipitation that in specific situation offers significant advantages. This process occurs at room temperature and totally in organic solvents. Mathiowitz et al. used this method to develop an improve technique of encapsulating insulin. In this example the polymer (polyanhydrides) was dissolved in methylene chloride, the desired amount of drug was added and then the mixture was suspended in silicone containing Span 85® and methylene chloride. After pouring the polymer solution into the silicone oil, petroleum ether was added and the mixture was stirred until the methylene chloride was extracted into the oil solution and sufficient microcapsule hardening was achieved. The resulting microcapsules were isolated by filtration, washed with petroleum ether and dried overnight under vacuum (Mathiowitz et al., 1988). Drug such as vancomycin was prepared by using poly (DL-lactide-co-glycolide) (PLGA) as polymer wall (Atkins, Peacock and Yates, 1998). Figure 8 shows a diagram of solvent removal technique.

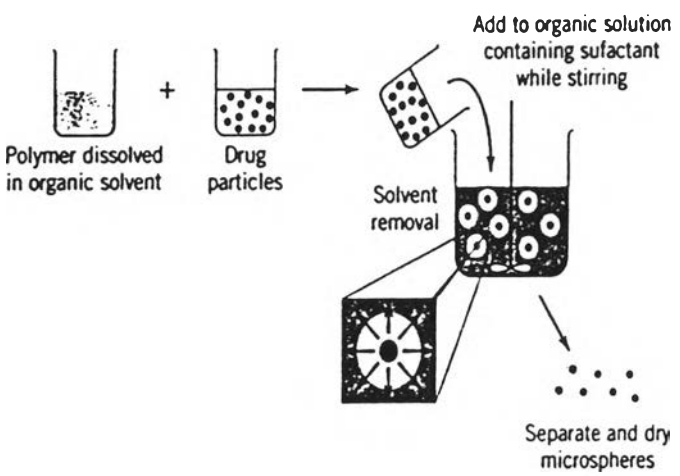


Figure 8 Microencapsulation by solvent removal technique (Mathiowitz, 1999).

2.7 Phase Separation

Pekarek et al. has developed a method to manufacture multilayered microcapsules in single step. This process combined the natural tendency of polymers to phase separation as solution concentration increase with the microencapsulation process of solvent evaporation. The basic differences between this process and organic phase separation or coacervation is that two polymers are used to cause the phase separation and that both are used in high concentration. Thus the final product, unlike coacervation, is the formation of two layers each containing two separate polymers. Example of polymers used by this technique were poly (L-Lactic acid –co-L-lysine) (PLLA) and poly (carboxyphenoxypropane-co-sebacic-acid), Poly (CPP-SA). In vitro and in vivo degradation of these double-walled microscapsules were studied. Degradation of multilayered microcapsules could serve as complex delivery vehicle for therapeutic agents and aid in the development of a useful delivery system (Pekarek, Jacob, and Mathiowitz, 1994).

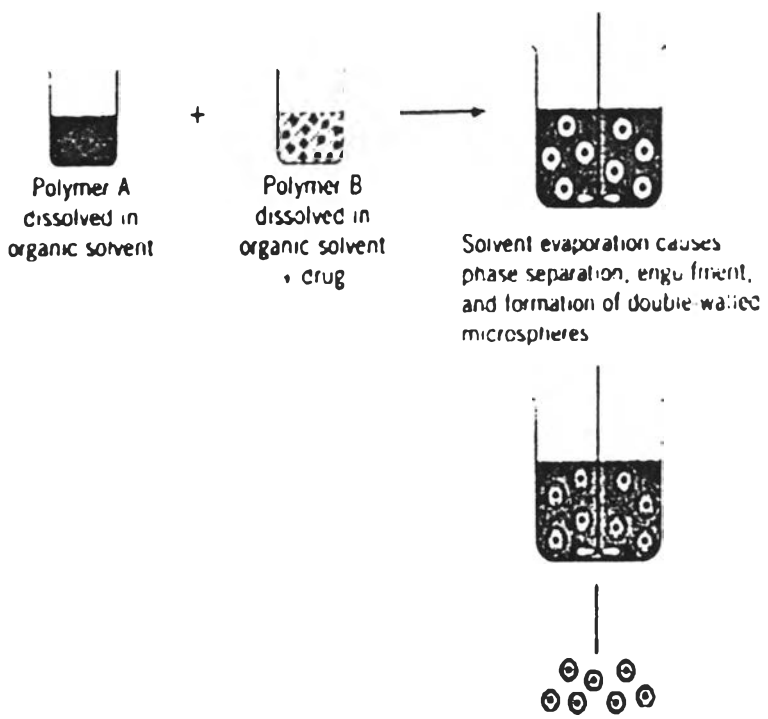


Figure 9 Microencapsulation by phase separation technique (Mathiowitz, 1999).

2.8 Hot melt

Hot melt is a process developed in the 1970s for photographic application. The melted polymer is mixed with drug that can be encapsulated as solid or liquid particles. The mixture is then suspended in immiscible solvent that is heated to 5 °C above the melting point of the polymer and stirred continuously using an overhead stirrer. Once the emulsion is stabilized, it is cooled until the core material has solidified. The solvent used in this process could be silicone and olive oil. Polyanhydride and poly (CPP-SA) are used as polymers and insulin has been encapsulated by this method (Mathiowitz and Langer, 1987).

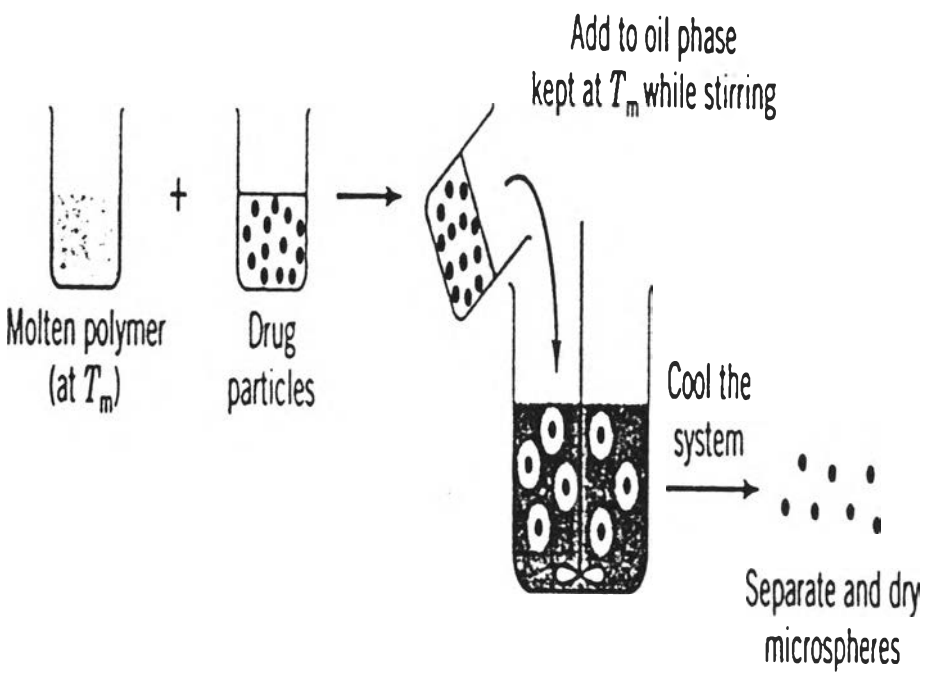


Figure 10 Microencapsulation by hot melt (Mathiowitz, 1999).

2.9 Spray Drying

Spray- drying is a method for preparing microcapsules or microspheres that is reproducible, rapid and easy to scale up. A drug or core material is dispersed in a coating solution and then the mixture is atomized into a hot air stream to remove the solvent from the coating material. Some examples of microencapsulation by spray drying with hydrophilic and hydrophobic polymer were studied as shown in table 2. The spray drying apparatus is illustrated in figure 11.

Table 2 Example of polymers used in microencapsulation by spray drying technique

Core	Water polymer /solution	Drying Temp(°C)	Size (μm)
Liquid paraffin	acetyl cellulose/acetone	130	50
Mineral oil	Zein/methanol	130	1-60
Ascorbic acid	lactose ethylcellulose/water	180	200
Titanium dioxide	polyacrylic acid/water	50	2

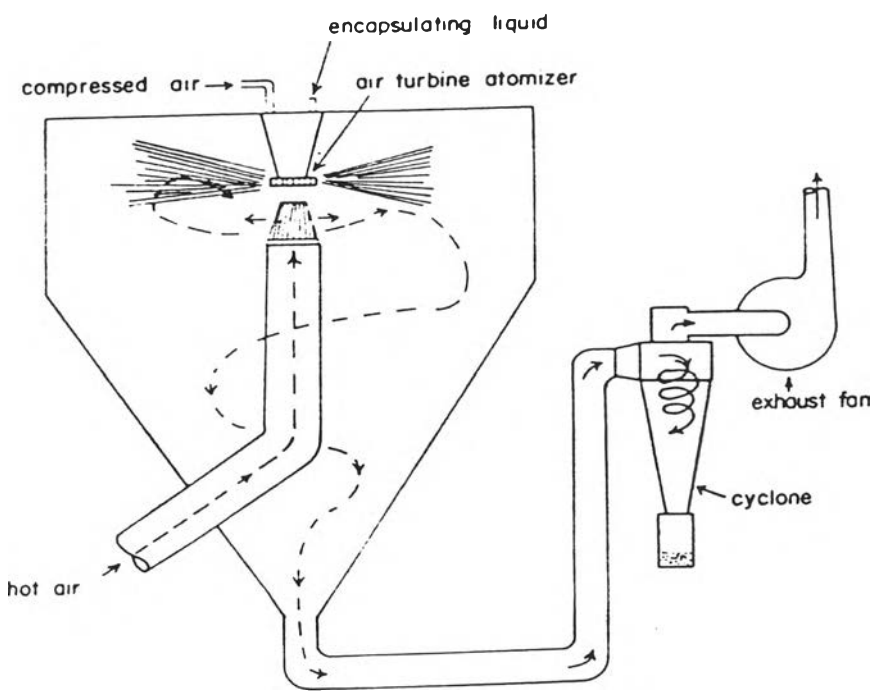


Figure 11 Diagram of spray dry apparatus.

3. Emulsion Formation

The first step in almost any encapsulation technique (described in table 1) involves the formation of an emulsion, usually of a polymeric solution inside a continuous phase. In order to disperse the drug inside this polymeric solution, emulsion must be created. Thus, an understanding of the properties of emulsion is extremely important. The emulsion determines the resulting particle size in final process of encapsulation. A suitable surfactant is need to process a stable emulsion, a result achieved by lowering the surface tension (r , usually from 40 to 5 mN/m^{-1}) . For pharmaceutical applications surfactants must acceptable for therapeutic use.

Many devices have been designed to produce emulsion. Depending on the desired particle size, a range of devices from simple stirrers or impellers to more sophisticated devices such as homogenizers, ultrasonic power generator or roller mills are available. The techniques and equipment used to form emulsion are variable for example shaking, pipe flow, injection, ultrasonic and aerosol etc.

4. D-panthenol

D-panthenol is the active alcohol analogue of D-pantothenic acid (vitamin B5), a vitamin of B- complex group that is a normal constituent of skin and hair. When applied topically, D-panthenol is converted to pantothenic acid. The skin has a relatively high requirement for co enzyme A, the biologically active form of D-panthothenic acid.
It is essential for normal functioning of epithelial tissues, and is a natural constituent of healthy skin. Skin manifestations of pantothenic acid deficiency are well known, and include cornification, depigmentation and desquamation. Cellular regeneration is accelerated by a topical application of D-panthenol, resulting in the documented healing properties of this provitamin (Idson, 1993).

Panthenol is available in two forms, namely, the dextrorotatory isomer, D-panthenol, or the racemic form and DL- panthenol (mixture of D-panthenol and L-panthenol). Due to the fact that only D-panthenol is converted to Vitamin B5 and not L-panthenol, the racemic DL-panthenol has only half-physiological activity of D-panthenol. D-panthenol is a colorless, viscous liquid, while DL-panthenol is a white

crystalline powder. Both forms are very soluble in water phase of products. Aqueous solutions of D-panthenol and DL-panthenol are most stable in the pH range 4 to 7, the optimum pH being approximately 6. Hydrolysis occurs when increasing rate at the pH varies from the optimum pH.

D-panthenol in skin care

In skin D-panthenol has the following properties:

- It improves and increases the humidity of the skin (moisturizing effect)
- It also makes dry skin softer and more elastic
- It has an anti-inflammatory effect and soothes irritated skin
- It stimulates epithelisation and help to heal minor wounds (shaving, skin grazes and blisters)

Weiser and Erelemann (1987) have shown that even low concentration of D-panthenol (0.1-2.5%) have a positive influence on epithelisation. The studies were carried out using a commercially available w/o cream with varying concentrations of D-panthenol.

D-panthenol in haircare

In haircare D-panthenol has the following properties:

- Give the hair long lasting moisturisation
- Improve the manageability of the hair
- Reduce the formation of split ends
- Thicken the hair
- Give the hair shine

D-Panthenol in nail care

The elasticity of fingernails depends on the water storage capacity of the nail keratin. D-panthenol can substantially increase the water storage capacity of the nails and by this mechanism the flexibility and stability of the nails is improved.

D-panthenol is available from a number of companies D-panthenol, DL-panthenol, D-calcium pantothenate, Ethyl panthenol and D-pantothenyl ethyl ether are offered for used as a hair and skin moisturizer. The recommended concentration in skin care is 0.5-5%, haircare 0.1-5% and nailcare 1%.

D-panthenol Microencapsulation

Interfacial polymerization is the selected method where two reactive monomers were dissolved in immiscible solvents, one in aqueous phase with D-panthenol reacted to the other monomer in solvent to form a polymeric membrane at interface of the w/o emulsion. The interfacial reaction occurred rapidly and therefore considered a rapid of preparing D-panthenol microcapsules. Proteins such as bovine serum albumin, ovalbumin and gelatin are investigated as wall materials and terephthaloyl chloride is used in this preparation.