CHAPTER II

LITERATURE REVIEW

A. Hemorrhoid

Hemorrhoids are cushions of tissue and varicose veins located in and around the rectal area. Hemorrhoids are a common condition but their true prevalence is unknown. Hemorrhoids may result from straining to move stool. Other contributing factors include pregnancy, aging, chronic constipation or diarrhea (Medical Resource, 2002).

1. Anatomy of hemorrhoid

The hemorrhoidal cushions appear predictably in the right anterior, right posterior and left lateral positions, although there may be intervening secondary hemorrhoidal complexes. The blood supply is similarly constant, deriving from the superior rectal artery, a branch of the inferior mesenteric artery, the middle rectal arteries arising from the internal iliac arteries; and the inferior rectal arteries arising from the pudendal arteries. The venous drainage transitions from the portal venous system above the level of the dentate line to the systemic venous system below this level.

2. Pathophysiology (Jay, 1996)

The hemorrhoid originates at the top of the anal canal, it is referred to as an internal hemorrhoid. If it originates at lower end of the anal canal near the anus, it is referred to as an external hemorrhoid (Figure1). Technically, the differentiation between internal and external hemorrhoids is made on the basis of whether the hemorrhoid originates above or below the dentate line.

Symptoms from external hemorrhoids typically result from acute thrombosis. The rapid tissue expansion produced by thrombosis and edema causes pain. Treatment in the acute setting often requires local excision. If symptoms have stabilized or are improving at the time of presentation, non-operative care, including stool-bulking agents, topical agents, and analgesics is indicated. If not treated in 2 to 4 weeks, the clot in the thrombosed vessels will either spontaneously drain through

the thinned overlying skin or be gradually reabsorbed, and the discomfort will gradually diminish. After resolution, redundant anal skin may remain as a perianal skin tag.

Internal hemorrhoids are located proximal to the dentate line and are covered by mucosa. Based on size and clinical symptoms, internal hemorrhoids can be further subdivided by grades or degrees:

- First-degree hemorrhoids: Hemorrhoids that bleed but do not prolapse.

- Second-degree hemorrhoids: Hemorrhoids out of the anal canal with bowel movements or straining but spontaneously reduce.

- Third-degree hemorrhoids: In chronic hemorrhoidal disease, the persistent prolapsing produces dilation of the anal sphincter and the hemorrhoids protrude with minimal provocation and usually require manual replacement.

- Fourth-degree hemorrhoids: Hemorrhoids are prolapsed out of the anus and cannot be reduced. Symptoms typically relate to mucosal protrusion, bleeding, perianal itching and discomfort, difficulties with perianal hygiene, and pain. Hemorrhoids that remain prolapsed develop ischemia, thrombosis, or gangrene.

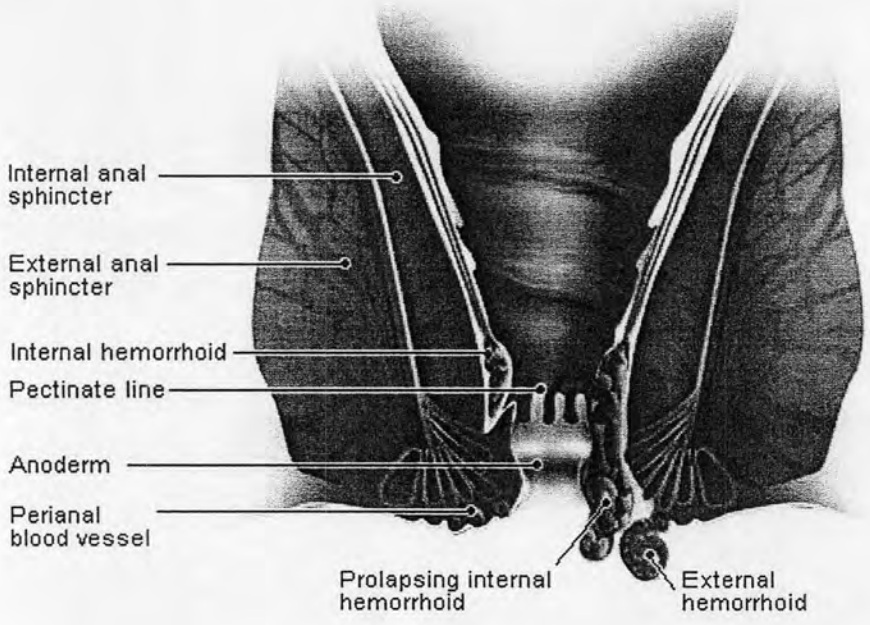


Figure 1 Formation of hemorrhoids

3. Treatments of hemorrhoid (Brian and Charles, 2004)

The treatments can be grouped into conservative (diet and vascular tonification), nonexcisional (sclerotherapy, rubber band ligation, cryotherapy, photocoagulation and electrocautery) and surgical methods (hemorrhoidectomy). First-degree hemorrhoids can be treated with medical management alone or with one of several non-operative outpatient therapies. Second-degree and relatively small and third-degree hemorrhoids can be treated with non-operative therapy. Surgery is generally reserved for the minority of patients who have large third-degree or fourth-degree hemorrhoids, acutely incarcerated and thrombosed hemorrhoids, hemorrhoids with an extensive and symptomatic external component, or patients who have undergone less aggressive therapy with poor results (Robert and James, 2004).

3.1 Non-operative treatment

3.1.1 Stool-bulking agents, behavior modification, and topical agents

Behavior modification also plays a key role in the initial management of patients with symptomatic hemorrhoidal disease. Prevention of straining and avoidance of precipitating factors that may cause excessive straining should be encouraged. Advice regarding perianal hygiene may be helpful in symptomatic relief and prevention of perineal dermatitis. Sitz baths and warm soaks should be encouraged as needed. Various topical creams, ointments, lotions, and suppositories have been developed with the intent of improving symptoms related to hemorrhoidal disease.

3.1.2 Sclerotherapy

Sclerotherapy is one of the oldest forms of treatment. During sclerotherapy, a liquid (phenol or quinine urea) is incorporated into the base of the hemorrhoid. Symptoms of hemorrhoid frequently return after several years and may require further treatment. Complications are usually related to incorrect placement or excess injection of sclerosing agent. The most common problem seen is superficial sloughing of the hemorrhoidal mucosa, which generally heals without treatment. Rarely, excessive sloughing can lead to scarring and stricture. Repetitive sclerotherapy treatments are not advised for this reason.

3.1.3 Rubber Band Ligation (RBL)

The principle of ligation with rubber bands is to encircle the base of the hemorrhoid anal cushion with rubber band. The tissue cut off by the rubber band dies and is replaced by an ulcer that heals with scarring. The most common complications of ligation are pain and bleeding one or two weeks. Bacteria infection may begin in the tissue surrounding the anal canal (cellulites). Symptoms frequently recur several years but usually can be treated with further ligation.

3.1.4 Cryotherapy

Cryotherapy uses cold temperatures to obliterate the veins and cause inflammation and scarring. It is more time consuming, associated with more post-treatment pain, and is less effective than other treatments. Therefore, this procedure is not commonly used.

3.1.5 Infrared photocoagulation (IRC)

A newer technique to treat internal hemorrhoids, an infrared coagulator delivers a controlled amount of infrared energy that penetrates the tissue to a predetermined level and is converted to heat. The physics of the energy are such that the majority of energy is deposited into the submucosa, producing inflammation, protein coagulation, tissue destruction, and eventual scarring. Immediately after coagulation, the tissue appears white. Over the next week, a dark eschar forms, eventually leaving a puckered pink to red scar. Disadvantages of this technique include the cost of the instrument, which is significantly higher than that of a rubber band ligator, and the limited effectiveness in treating large, bulky hemorrhoids. Also, the development of anal fissures has been reported after IRC in rare cases.

3.1.6 Electrocautery

Bipolar and direct current devices are currently available for electrocautery. Bipolar diathermy uses a bipolar radio frequency electrical current to generate a coagulum of tissue at the end of a cautery tipped applicator. A 2-second pulse is applied to the base of each hemorrhoid. Direct current therapy uses a special probe to deliver an electrical current of up to 16 milliamperes to the internal hemorrhoid bundle, for a period of up to 10 minutes. This utility of this technique is limited by patient discomfort.

3.2 Operative treatment

Hemorrhoidectomy, surgical removal of hemorrhoids usually is reserved for patients with third or fourth degree of hemorrhoids. However non-operative treatments are preferred because it is associated with less pain and fewer complications than operative treatment.

A major component of a safe and effective therapy for hemorrhoids is the use of botanical and nutritional therapies. Several botanical extracts such as *Centella asiatica* have been shown to improve microcirculation, capillary flow, and vascular tone, and strengthen connective tissues of the perivascular amorphous substrate (Mackay, 2001). The goals of botanical and nutritional support are consistent with the philosophy of treating the cause of a disease. Conversely, the bulk of standard treatments for hemorrhoids are geared toward removing the problem or palliating the disease. Additionally, the low compliance associated with treatments such as hydrotherapy, mechanical compression therapy, and diet and lifestyle changes renders oral dietary supplementation an attractive option. The use of nutritional and botanical agents for the treatment of hemorrhoids is possibly the missing link to an effective conservative approach to these diseases. Early intervention with conservative therapies may prevent time-consuming and expensive complications of hemorrhoids

B. Botanical, Chemical and Pharmacological Aspects of *Centella asiatica* (Linn.)

1. Botanical aspects of *Centella asiatica* (Linn.)

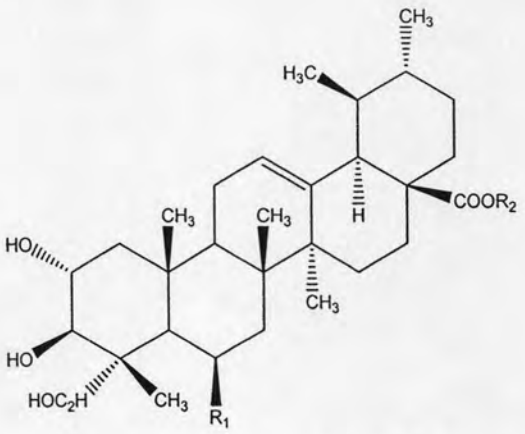
Centella asiatica (Linn.) is known as Bua-bok, Gotu Kola, Asiatic Pennywort, Indian Pennywort, Indian Water Navelwort, Mandukparni, etc. It is a cultivated plant in the family Apiaceae (umbelliferae), the synonym *Hydrocotyle asiatica* (Linn.), that creeping subtropical and tropical climates of Asia, Africa, North and South America.

Centella asiatica (Linn.) is a slender tailing herb, rooting at the nodes. The leaves are thin and soft, with palmate nerves, hairless or with only few hairs, kidney shaped about 2 to 5 cm in diameter. The stems (stolons) are slender, prostrate and often reddish colored. The flowers are pale violet. Each umbel bears 2 to 5 small oval fruits, enclosed within a thick, hard pericarp. The aerial plants are harvested throughout the year (Figure 2).

Figure 2 *Centella asiatica* (Linn.) Urban

2. Chemical components of *Centella asiatica* (Linn.)

Centella asiatica contains a wide range of substances. The substances of therapeutic interest are the saponin-containing triterpene acids and their sugar esters. The major triterpenoid glycosides are asiaticoside, madecassoside, brahmoside, brahminoside and asiatic acid, madecassic acid are presented together with their glycosides (Figure 3).



- Asiaticoside (R_1 = H R_2 = glu-glu-rham)
- Madecassoside (R_1 = OH R_2 = glu-glu-rham)
- Brahmoside (R_1 = OH R_2 = rham-glu-arab)
- Brahminoside (R_1 = OH R_2 = 2 glu-rham-arab)
- Asiatic acid (R_1 = H R_2 = H)
- Madecassic acid (R_1 = OH R_2 = H)

Figure 3 Some structures of the triterpenoids from *Centella asiatica* (Linn.)

3. Pharmacological activities of *Centella asiatica* (Linn.)

From in vitro study, topical application of asiaticoside significantly enhanced the rate of wound healing as assessed by an increase in collagen synthesis and tensile strength of the wound tissues (Shukla et al., 1999). Maquart et al. (1990) also reported elevated collagen synthesis in fibroblasts by asiaticoside. Rosen, Blumenthal and McCallum (1972) also reported that asiaticoside promoted wound healing in rats and significantly increased the tensile strength of newly formed skin. The European Agency for the Evaluation of Medicinal Products Veterinary Medicines Evaluation unit (1998) reported that madecassic acid, asiaticoside and asiatic acid acted on fibroblast cells and equilibrated collagen fiber synthesis. The overall effects contributed to the restoration of elastic connective tissue, a reduction in fibrosis and a short in the time necessary for wound healing.

For most clinical studies of *Centella asiatica* used either undefined alcohol or aqueous extracts or one of the following extracts; TECA, TTFCA, or TTF. The extracts TECA (titrated extract of *Centella asiatica*) and TTFCA (total triterpenoid fraction of *Centella asiatica*) are combinations comprised of asiatic acid (30%), madecassic acid (30%), and asiaticoside (40%). The centella extract TTF (total triterpenic fraction) is comprised of asiatic acid and madecassic acid (60%) in a ratio not clearly defined, in combination with asiaticoside (40%) (Brinkhaus et al., 2000). Rigorous clinical investigations of *Centella asiatica* have been conducted on chronic venous insufficiency and varicose veins. Centella has the potential to enhance connective tissue integrity, elevate antioxidant levels in wound healing, and improve capillary permeability (Shukla, Rasik and Dhawan, 1999; Belcaro, Grimaldi and Guidi, 1990).

A randomized, multicenter, placebo-controlled, double-blind study investigated centella extract in the treatment of venous insufficiency. Ninety-four patients received either TECA in two different doses (120 mg/day; 60 mg/day) or placebo over a two-month period. Results were evaluated subjectively by the patients' symptoms and objectively by plethysmography. The TECA groups resulted in significant improvements ($P<0.05$) in symptoms of heaviness in the lower limbs, edema, and overall evaluation by the patient. Venous distensability, measured by a mercury strain gauge plethysmograph at three occlusion pressures, was improved for

the TECA groups but aggravated for the placebo group (Pointel et al., 1987). The differences in the effect of the different TECA doses were not significant, but did reveal a dose-effect relationship.

Mucopolysaccharides are one of the main components of the amorphous cellular matrix (ground substance) that maintains vascular integrity. The biochemical action of centella extract was shown to reduce serum levels of lysosomal enzymes involved in the degradation of mucopolysaccharides. The TTFCA extract was administered (30 mg three times daily) to 20 patients with severe varicose veins in the leg over an observation period of three months. Prior to the treatment, elevated baseline serum lysosomal enzymes were established (beta-glucuronidase 1.8 +/-0.4 microM/min/L, beta-N-acetylglucosaminidase 23.1+/-0.4 microM/min/L, and arysulfatase 0.078+/-0.003 microM/min/L) indicating an increased muco-polysaccharide turnover in subjects with varicose veins. During the treatment period these levels fell progressively. At the end of the three-month trial there was a significant reduction in the serum levels of the lysosomal enzymes (betaglucuronidase 1.2 +/- 0.05 microM/min/L, beta-N-acetylglucosaminidase 17.7+/- 0.7 microM/min/L, arysulfatase 0.042+/-0.003 microM/min/L). These reductions were interpreted as evidence of a positive effect of the TTFCA extract on the pathogenesis of varicose veins (Arpaia et al., 1990).

In a double-blind, placebo-controlled study the effects of centella extract on capillary filtration rate was investigated. *Centella asiatica* (TTFCA) extract was administered to 62 patients at two different doses (90mg/day; 180mg/day). Capillary filtration rate was evaluated in comparison to placebo. At the end of the four-week treatment period there was a dose-dependent reduction in capillary filtration rate measured by plethysmography. In comparison with the placebo group, the dose dependent improvements seen in the TTFCA group were significant. The reduced capillary filtration rate was associated with improvement in microcirculation and in clinical symptoms (Belcaro, Grimaldi and Guidi, 1990; Belcaro, Rulo and Grimaldi, 1990).

In addition, local application of TTFCA extract has been shown to improve vascular tone. In a double-blind study involving 80 patients, centella extract was applied locally three times daily to patients with various venous disorders (including

hemorrhoids and varicose veins). Patients, physicians, and ultrasonic examination noted subjective and objective improvements in symptoms (Allegra et al., 1981).

C. Mucoadhesive Chitosan Microspheres

1. Polymer: Chitosan

1.1 Chemical structure of chitosan

Chitosan, a nature linear biopolyaminosaccharide is obtained by alkaline deacetylation of chitin. Chitosan molecule is a copolymer of *N*-acetyl-D-glucosamine and D-glucosamine (Figure 4). The sugar backbone consists of β-1, 4-linked D-glucos-amine with a high degree of *N*-acetylation, a structure very similar to that of cellulose, except that the acetylamino group replaces the hydroxyl group on the C-2 position. Thus, chitosan is poly (*N*-acetyl-2-amino-2-deoxy-D-glucopyranose), where the *N*-acetyl-2-amino-2-deoxy-D-glucopyranose (or Glu-NH_2) units are linked by (1→4)-β-glycosidic bonds.

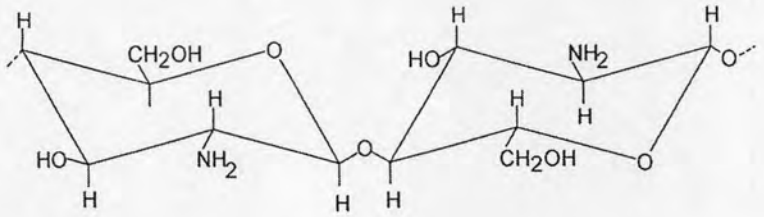


Figure 4 Structure of chitosan

1.2 Availability of chitosan

Chitosan obtained by deacetylation of chitin. Chitin is the second abundant polysaccharide next to cellulose. Chitin is the principle component of protective cuticles of crustaceans such as crabs, shrimps, prawns, lobsters and cell wall of some fungi such as *aspergillus* and *mucor*. Commercially, chitosan is available in the form of dry flakes, solution and dry powder.

1.3 Physicochemical and biological properties of chitosan

Chitosan has an average molecular weight ranging between 3,800 and 2,000,000 and is from 66 to 95% deacetylated (Kas, 1997). Particle size, density, viscosity, degree of deacetylation and molecular weight are important characteristics of chitosan which influence the properties of pharmaceutical formulations based on chitosan. The pharmaceutical requirements of chitosan are; particle size < 30 μm, density between 1.35-1.40 g/cm^3, pH 6.5-7.5.

Chitosan is a weak base with a pK_a value of the D-glucosamine residue of about 6.2-7.0 and is insoluble in water and organic solvents. However, it is soluble in dilute aqueous acidic solution (pH < 6.5). Chitosan has low oral toxicity with an LD_{50} in rats of 16 g/kg. Toxicity of chitosan might depend on different factors such as degree of deacetylation, molecular weight, purity and route of administration (Knapczk et al., 1984)

Chitosan possesses -OH and -NH_2 groups that can give rise to hydrogen bonding and the linear molecule expresses a sufficient chain flexibility, the conformation of which is highly dependent on ionic strength. These properties are considered essential for mucoadhesion (Peppas and Buri, 1985). Furthermore, the cationic polyelectrolyte nature of chitosan could provide a strong electroststic interaction with mucus or negatively charged mucosal surface (Figure 5). Due to molecular attractive forces formed by electrostatic interaction between positively charged chitosan and negatively charged mucosal surfaces (Sinha et al., 2004). Mucoadhesive properties offer various advantages such as (Hejazi and Amiji, 2003):

(a) Longer residence time of the dosage form on mucosal tissues. This will improve absorption of the drug and increase the drug's bioavailability.
(b) Higher drug concentration at the site of adhesion-absorption.
(c) Immediate absorption from the bioadhesive drug delivery system without previous dilution and possible degradation in the luminal fluids.
(d) Enhancement of topical action of certain drugs.

The importance of the mucoadhesive properties of chitosan has been demonstrated in earlier work such as; using chitosan delivery peptide drugs by nasal route (Illum, Farraj and Davis, 1994), enhance absorption insulin on nasal membrane (Aspden, Illum and Skaugrud, 1996) and the study of drug release from microspheres adhered on pig vesical mucosa (Burjak et al., 2001).

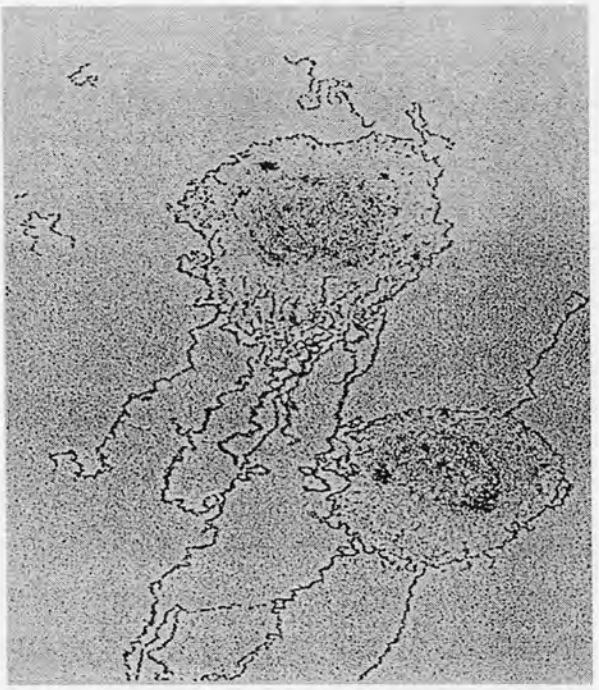


Figure 5 Interaction of mucin with chitosan; showing mucin as long strands attached to chitosan aggregates. Chitosan interacts with mucin through charge interaction and hydrogen-bonding mechanisms (Stanley, 2005).

So, properties such as biodegradability, low toxicity, good biocompatibility and mucoadhesiveness make chitosan suitable for use in biomedical and pharmaceutical formulations.

2. Microspheres: spray drying

2.1 Terminology of microspheres

The terminology used to describe microparticulate formulations can sometimes be inconsistent and confusing to readers unfamiliar with the field. Essentially, the term "microparticle" refers to a particle with a diameter of 1–1000 μm,

irrespective of the precise interior or exterior structure. Within the broad category of microparticles, "microspheres" specifically refers to spherical microparticles and the subcategory of "microcapsules" applies to microparticles which have a core surrounded by a material which is distinctly different from that of the core. The core may be solid, liquid, or even gas. Despite the specific and logical subcategories, many researchers use the terms interchangeably, often to the confusion of the reader. It is usually assumed that a formulation described as a microparticle is comprised of a fairly homogeneous mixture of polymer and active agent. Some variations on microparticle structures are given in Figure 6. As the domains and subdomains of active agent within microcapsules become progressively smaller, the microcapsules become microparticles (Birnbaum and Peppas, 2003).

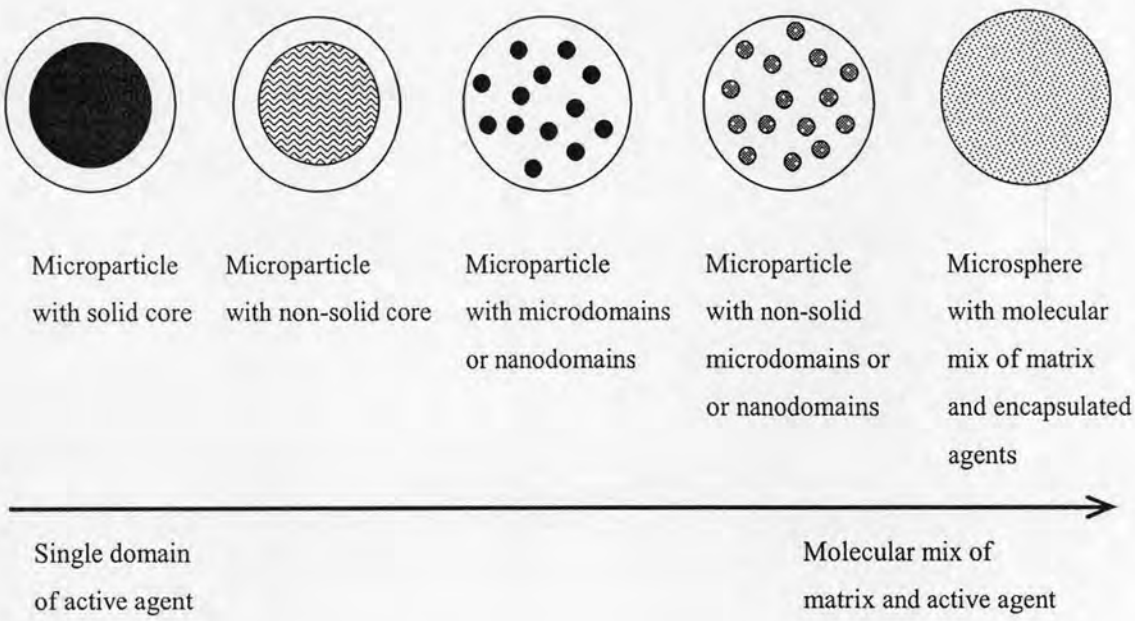


Figure 6 Variations of microparticle formulations.

The use of microsphere-based therapy allows drug release to be carefully tailored to the specific treatment site through the choice and formulation of various drug-polymer combinations. The total dose of medication and the kinetics of release are the variables, which can be manipulated to achieve the desired result. Microspheres can be developed into an optimal drug delivery system which will provide the desired release profile. Microsphere-based systems may increase the life span of active constituents and control the release of bioactive agents. Being small in

size, microspheres have large surface to volume ratios and can be used for controlled release of insoluble drugs.

Extensive research is being carried out to exploit chitosan as a drug carrier to attain the desirable drug release profile. Chitosan microspheres are used to provide controlled release of many drugs and to improve the bioavailability of degradable substances such as protein or enhance the uptake of hydrophilic substances across the epithelial layers. These microspheres are reported both for parenteral and oral drug delivery. Chitosan has also been used as a potential carrier for prolonged delivery of drugs (Tozaki et al., 1997), macromolecules and targeted drug delivery; (Lee et al., 1998).

2.2 spray drying process

Chitosan microspheres can be prepared by spray drying. Spray drying is extensively used in the pharmaceuticals to produce dry powders, granules or agglomerates from drug-excipient solutions and suspensions. This technique transforms liquid feed into dry powder in a one step, continuous particle processing operation and can be applied to a wide variety of materials. The spray drying process encompasses the following four stages (Master, 1979):

(a) Atomization of the feed into a spray

(b) Spray-air contact

(c) Drying of the spray

(d) Separation of the dried product from the drying gas

Spray drying involved evaporation of moisture from an atomized feed by mixing the spray and the drying medium. The drying medium is typically air. The drying proceeds until the desired moisture content is reached in the sprayed particles and the product is then separated from the air. So, the process involves assessing of technological parameters as follows: concentration of the medium to be sprayed, inlet and outlet temperature, feed rate, air flow rate, heating and exhausting.

2.2.1 Effect of processing and formulation parameters on the properties of spray dried

Wan, Heng and Chia (1991) prepared coated theophylline particles by a spray drying process. This process was carried out using an aqueous solution of hydroxypropyl methylcellulose (HPMC), 52.2 cps, as the coating polymer. The

effects of spray nozzle size, inlet drying temperature, drying air flow rate, feed spray rate and atomizing pressure were studied. The flow properties of the spray-dried particles improved with decrease in the air-to-liquid diameter ratio of the nozzle and increase in the inlet temperature. A high inlet drying temperature produced coated particles with a slower drug dissolution rate. The particles that had been spray dried at a faster drying air flow rate were found to have better flowability. High feed spray rates resulted in ineffective atomization, producing badly formed spray-dried products. Atomizing pressure affected only the particle size of the product formed.

Stahl et al. (2002) investigated the effect of process variables on the degradation and physical properties of spray dried insulin intended for inhalation. The effects of feed flow rate, nozzle gas flow rate, inlet air temperature and aspirator capacity on the degradation and physical properties of spray dried insulin intended for inhalation have been evaluated by factorial experimental design. The nozzle gas flow rate was not to influence the degradation of insulin. The yield increased when the nozzle flow was decreased. Decreased nozzle flow decreases the atomization energy and thus producing enlarged droplets. These droplets dry to larger particles, which are more easily captured through the centrifugal force in the cyclone. Increased aspirator capacity and increased inlet air temperature both resulted in decreased moisture content because of increased supplies of heat energy allows a more efficient drying. The correlation between moisture content and outlet air temperature is in agreement with previous studies that spray-dried β-galactosidase (Broadhead et al., 1994). Increased feed flow reduced the outlet air temperature, resulting in lower drying capacity and thus higher moisture content in the powder product. Increased inlet air temperature increased the particle size which has also been observed before by Broadhead et al. (1994)

2.2.2. Advantages of spray drying (Master, 1979)

a) Spray drying is a single-step operation from liquid feed to dry product. Frequently this eliminates such step as precipitation or crystallizing, centrifuging or filtering, grinding and dust collecting operations associated with them.

b) The process is continuous, although it can operate with feed from a prior batch process.

c) Adaptable to full automatic control.

d) Dried product specifications meet through dryer design and operational flexibility:

- Required product form (particle as spheres, fines and agglomerates)
- Required properties (dusty or dustless, degree of flowability, wettability and etc.)

e) Applicable to both heat sensitive and heat-resistant materials.

f) Feedstocks in solution, slurry, thixotropic paste or melt form can be handling, if pumpable.

g) Corrosive and abrasive feed stocks can be readily handled.

h) Corrosion is reduced or prevented because the material does not contact the equipment surfaces until it is dry. This permits selection of lower-cost materials of construction.

i) Maintenance costs are low because there is few moving part.

j) Labor costs are low because only one operator is required, even on large installations. Because the evaporation usually is done under slight vacuum, it is easy to keep the equipment and area clean.

k) Operator requirements are the same for both small and large dryers, hence spray drying is basically a high-volume system with low labor cost.

l) Spray drying is an airborne process; hence there is very low material holdup in the equipment.

m) Designs are available to handle:

- Evaporation of organic solvents without explosion/fire risks.
- Powders that form potentially explosive mixtures in air.
- Products that create odor during drying.
- Toxic products.
- Products requiring aseptic/hygienic drying conditions.

D. *In situ* Forming Gel (Temperature Sensitive System): Poloxamer

In situ forming systems have been reported for various biomedical applications, including drug delivery, cell encapsulation, and tissue repair. These systems are injectable fluids that can be introduced into the body in a minimally invasive manner prior to solidifying or gelling within the desired tissue, organ, or body cavity. Injectable gel-forming matrices offer several advantages over systems

shaped into their final form before implantation. For example, injectable materials do not require a surgical procedure for placement (and withdrawal if not biodegradable), and various therapeutic agents can be incorporated by simple mixing. When they are used to fill a cavity or a defect, their flowing nature enables a good fit.

In situ formation can occur as a result of either a physical or chemical change of the system. The ideal system would be a solution that is a free-flowing, injectable liquid at ambient temperature. It should then gel at body temperature with minimal syneresis. Moreover, loading with drugs or cells should be achieved by simple mixing.

In situ forming systems that do not require organic solvents or copolymerization agents have gained increasing attention. They are liquid aqueous solutions before administration, but gel under physiological conditions. Gelation can occur *in situ* by ionic cross-linking (Rozier et al., 1989; Cohen et al., 1997) or after a change in pH (Kumar, Haglund and Himmelstein, 1994; Srividya, Cardoza and Amin 2001) or temperature. For this study, using poloxamer, it exploits temperature-induced phase transition.

1. Chemical structure of poloxamer

The poloxamers consist of more than 30 different non-ionic surface-active agents. These polymers are ABA-type triblock copolymers composed of PEO (A) and PPO (B) units [PEO-PPO-PEO] (Figure 7).

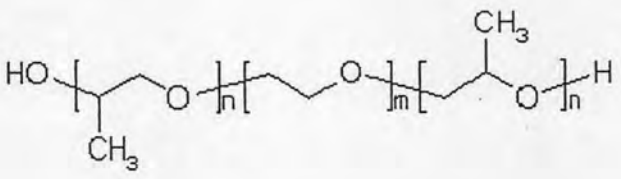


Figure 7 Structure of poloxamer

2. Physicochemical and biological properties of poloxamer

Poloxamer series covers a range of liquids, pastes, and solids, with molecular weights and ethylene oxide - propylene oxide weight ratios varying from 1,100 to 14,000 and 1:9 to 8:2, respectively. The poloxamers are widely used in industry.

With their surfactant properties, they have applications in detergency, dispersion, stabilization, foaming, and emulsification. The requirements of each application can be monitored by the architecture of the triblock, i.e., molecular weight and relative size block.

Poloxamer 407 is usually regarded as non-toxic. After intramuscular injection in rabbits, poloxamers 238 and 407 displayed musculoirritancy/toxicity comparable to that of traditional intramuscular vehicles, such as saline and peanut oil (Johnston and Miller, 1985). However, other studies have reported systemic side effects. Wout et al. (1992) demonstrated that poloxamer 407 injected intraperitoneally into rats (1.5 g/kg) resulted in sustained hypercholesterolemia and hypertriglyceridemia (>96 h). Palmer, Emeson and Johnston (1998) obtained similar results in mice. It was suggested that the predominant mechanism for this effect was inhibition of heparin-releasable lipoprotein lipase (Johnston and Palmer, 1993). Blonder et al. (1999) examined whether lower but clinically useful doses of poloxamer 407 gels induced hyperlipidemia in rabbits. The highest dose (137.5 mg/kg) significantly increased serum triglycerides and cholesterol in both male and female rabbits, with peak values observed 2 days after injection. The lower doses (5.5–27.5 mg/kg) did not alter serum lipids. Hence, the amount of administered polymer should be kept to a minimum, especially when repeated dosing is required.

In aqueous solution, with increasing temperature, poloxamers aggregate in micelles to minimize the solution. The micellar behavior of poloxamers was first assessed using differential ultrasonic velocity and light scattering (Rassing and Attwood, 1983). Since then, some studies have been devoted to the analysis of the poloxamer micellization process. In the low temperature region, poloxamers exist as unimers. Upon warming, equilibrium between unimers and micelles is established, and finally aggregates are formed at higher temperatures. It is generally accepted that these micelles are spherical and consist of a PPO core with a PEO water swollen shell. This conformation is attributed to the fact that PPO is poorly water soluble and PEO is highly soluble in aqueous solvent (Zhou and Chu, 1988).

At sufficiently high concentrations, some poloxamer solutions showed reversible thermal gelation. The gels formed under these conditions are found to be stiff and clear. This phenomenon has been extensively studied (Vadnere et al., 1984;

Tung, 1994). However, several points such as the driving force of gelation and the crystallinity of the gel are still open to discussion.

Many results have prompted the use of poloxamer 407 in the design of medical, pharmaceutical and cosmetic systems. Some recent applications are reported in various administrations. For parenteral injection, poloxamer gels can prolong drug release compared to solutions, but the delivery period rarely exceeds a few days. This characteristic makes poloxamer gels interesting for short-term therapies like pain management (Paavola et al., 2000), infection treatment (Veyries et al., 1999; Zhang et al., 2002) and fertility control (Wenzel et al., 2002). Besides injectables, other administration routes have been evaluated, such as rectal (Choi et al., 1998; Ryu et al., 1999), vaginal (Chang et al., 2002), transdermal (Shin, Cho and Oh, 2000; Liaw and Lin, 2000) and ophthalmic (El-Kamel, 2002; Wei et al., 2002). Poloxamer formulations generally increased drug residence time at application sites, resulting in improved bioavailability and efficacy.

E. The Release Mechanism of Controlled Release System

Different mathematical models are used to describe the kinetics of the drug release process from the matrices; the most suited being the one which best fits the experimental results. The pattern of delivery achieves by a controlled release system can vary over a wide range, but most of all release profiles are categorized into three major types:

1. The zero-order model describes release from porous (erodible) matrices which the drug release rate is independent of its concentration (equation 1).
2. The first-order model describes the release in the system which the release rate is concentration dependent (equation 2).
3. The square root of time or Higuchi model describes release by Fickian diffusion through a porous matrix (equation 3).

1. Zero-order model

An ideal controlled release device is one which can deliver the drug at a constant rate until the device is exhausted of active agent. Mathematically, the release rate from this device is given as;

$$Q_t = Q_0 + k_0 t \qquad (1)$$

Q_t is the amount of drug release at time t

Q_0 is the initial amount of drug in sample at $t = 0$

k_0 is the release rate constant for zero-order

2. First-order model

The first order pattern is the second common type of the release model. The release rate in this case is proportional to the mass of active agent contained within the device. In first order model, therefore, the rate declined exponentially with time, approaching a release rate of zero as the device approached exhaustion. The rate is then given as;

$$Q_t = Q_\infty (1 - e^{-k_1 t}) \qquad (2)$$

Q_t is the amount of drug release at time t

Q_∞ is the total amount of drug in matrix

k_1 is the release rate constant for first-order

First order pattern can be predicted by plotting the logarithm of the percent of drug remaining against time. If it was first order model, linear relationship was obtained.

3. Square root of time model (Higuchi model)

The third common release pattern, referred to square root of time, provides compound release that is linear with the reciprocal of the square root of time. The release rate then given as (Higuchi, 1963);

$$Q_t = k_H t^{1/2} \qquad (3)$$

Q_t is the amount of drug release at time t

k_H is the release rate constant for Higuchi model

In contrast to first order release, the release rate here remained finite as the device approached exhaustion. The plot of amount of drug released from matrix versus the square root of time should be increased linearity if drug release from the matrix is diffusion controlled. Although the above equation is based on release from a single face, it may be used to describe diffusion-controlled release from all surface matrixes.