CHAPTER II

LITERATURE REVIEW

1. *Phyllanthus emblica*

P. emblica L. is a member of Euphobiaceae. *P. emblica* is a shrub or tree distributed in subtropical and tropical areas of Southeast Asia, particularly in China, India, Indonesia, The Malay Peninsula, Bangladesh, Nepal, Pakistan, Uzbekistan, Sri Lanka and Thailand (Scartezzini and Speroni, 2000, Zhang et al., 2001., Zhang et al., 2004). In Thailand, it is known as "Makhampom". The plant is a tree of small or moderate size with a greenish-gray bark and greenish-yellow flowers. The feathery leaves are linear-oblong, with a ronded base and obtuse or acute apex. The tender fruits are green, fleshy, globose and shining, and change to light yellow or brick-red when matures (Summanen, 1999). The fruit is similar in appearance to the common gooseberry, usually called the "Indian gooseberry" commonly edible and abundantly available fresh fruits of *P. emblica* are collected during the months of November to January (Ghosal, 1996).

The active substances of *P. emblica* are alkaloids, benzinoids, ascorbic acid, diterpenes, triterpenes, flavonoids, sterols and tannins. Chemical investigation of *P. emblica* led to the isolation of several novel sesquiterpenoids from the roots, new organic acid gallates and polyphenols from the fruit juice, and new ellagitannins and flavonoids from the branches and leaves. Some of these substances are estimated for their anti-proliferative activities and the antitumor activities of phonols might be linked to their anti-inflammatory properties (Jeena and Kuttan, 2001., Zhang et al., 2004). The methanolic extract (75%) of *P. emblica* was found to inhibit lipid peroxide formation and to scavenge hydroxyl and superoxide radicals *in vitro* (Suba and Kuttan, 2002).

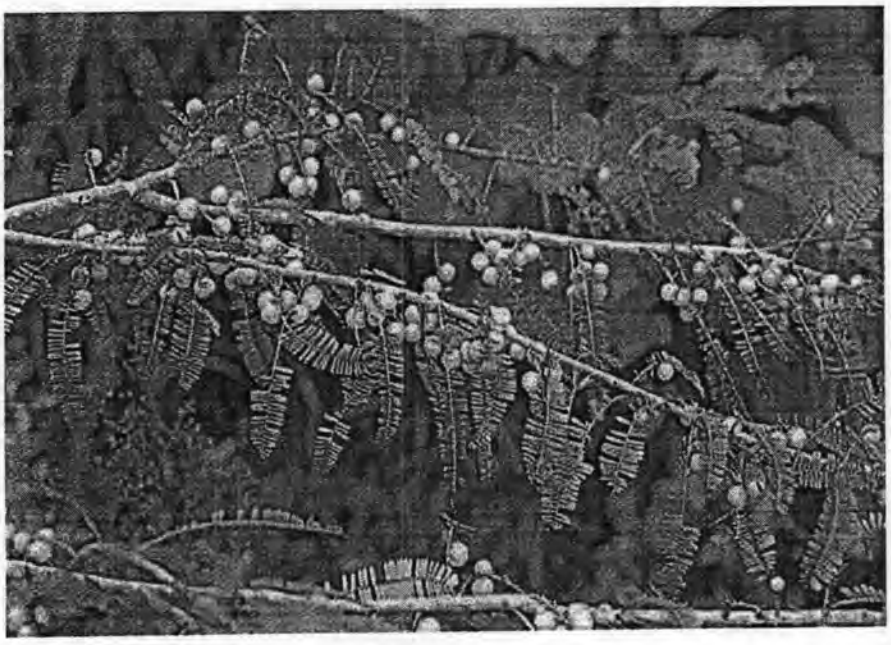

Figure 1 *Phyllanthus emblica* fruit

The fruit of *P. emblica* is the most commonly use plant part (Figure1), with the fresh fruit being preferred. The air-dried fruit is also used for the treatment of cancer in Tibetan and Egyptian medicines (Zhang et al., 2004). Recently, fruits have been tested for their antiviral activity particularly for inhibiting reverse transcriptase in the replication of retroviruses like HIV-1 (Scartezzini and Speroni, 2000).

P. emblica fruit extract has been shown to have multi-function benefits. Low molecular weight tannins (<1000), namely Emblicanin A and Emblicanin B, along with Pedunculagin and Punigluconin are the key ingredients of *P. emblica* fruit extracts (Chaudhuri et al., 2004). The tannin-based *P. emblica* extract has been found to have broad-spectrum antioxidant activity (Chaudhuri, 2002). This fruit also exhibits inhibitory effect on the activity of collagenase and stromelysin-1 (collagen-degrading enzymes), which is increased by UV light. Reduction in matrix-metallopreoteinase (MMPs) activity helps reduce wrinkling and loss of elasticity of skin. *P. emblica* thus helps younger looking skin and act as anti-ageing agent. Though its primary applications within the skincare industry are as a photo-protective agent and skin lightener, it offers a host of other benefits for the skin (Chaudhuri et al., 2004, Chaudhuri and EMD chemicals, Inc., 2004).

2. Tannin

Tannin is the group of phenolic compounds. Tradition use of tannins as plants for converting animal hides to leather (tainning), their ability to interact with and precipitate protein, including the proteins found in animal skin. The term tannin comes from the ancient Celtic word for oak, a typical source for tannins for leather making. Bate-Smith defined tannins as "water-soluble phenolic compounds having molecular weights between 500-3000, the usual phenolic reactions, special properties such as the ability to precipitate alkaloids, gelatin and other proteins. There are two groups in tannins, condensed tannins are polymeric flavonoids. The flavonoids are a diverse group of metabolites based on a heterocyclic ring system derived from phenylalanine and polyketide biosynthesis.

Hydrolysable tannins are derivatives of gallic acid (3, 4, 5-trihydroxyl benzoic acid). Gallic acid is esterified to a core polyol, and the galloyl groups may be further esterified or oxidatively crosslinked to yield more complex hydrolysable tannins (Hangerman 1998, 2002).

3. Gallic acid

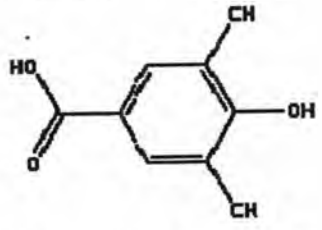

Figure 2 Structure of gallic acid

Gallic acid (Figure 2) is widespread in plant foods and beverages such as tea and wine was proven to be one of the anticarcinogenic polyphonols presented in green tea. The consumption of France of a diet high in saturated fat coupled with an apparently low incidence of coronary heart disease has been associated with the consumption of red wine. Antioxidants presented in red wine have been shown to have a protective role against oxidation of LDL in vitro. It is a strong chelating agent and forms complex of high stability with iron. It has shown phytotoxicity and

antifungal activity against Fusarium semitectum, F. fusiformis and Alternarea altternata. It is used for arteriosclerosis prevention. Otherwise, gallic acid has been used in pharmaceuticals industry (such as in the synthesis of Trimethoprim) in food and feed industries, in ink dyes, in laboratories for absorbing oxygen, in the production of azo dyes, and in photography (Polewski et al., 2002).

4. Folin-Ciocalteu reaction

The Folin-Ciocalteu method (Folin and Ciocalteu, 1927) was developed in 1927 and it originated from chemical reagents used for tyrosine analysis in which oxidation of phenols by a molybdotungstate reagent yields a colored product at 745-750 nm

$Na_2WO_4/Na_2MoO_4 \longrightarrow \cdot(\text{phenol-MoW11O40})^{-4}$

Mo (VT) (yellow) + e- $\longrightarrow$ Mo (V) (blue)

This method was official method of the Association of Analytical Communities (AOAC). Folin-Ciocalteau procedure is based on the reductive power of aromatic hydroxyls with the phosphomolybdate comples of the Folin-Ciocalteu reagent (phosphomolybdate and phosphotungstate). This method measures the total hydroxyl groups of phenolic compounds. The absorbance is obtained by spectrophotometer reading at 747 nm. The concentration of the total phenolic content is determined by comparison with the optical density values of different concentrations of a standard phenolic compound of gallic acid equivalent (Sato et al., 1996)

5. Liposomes

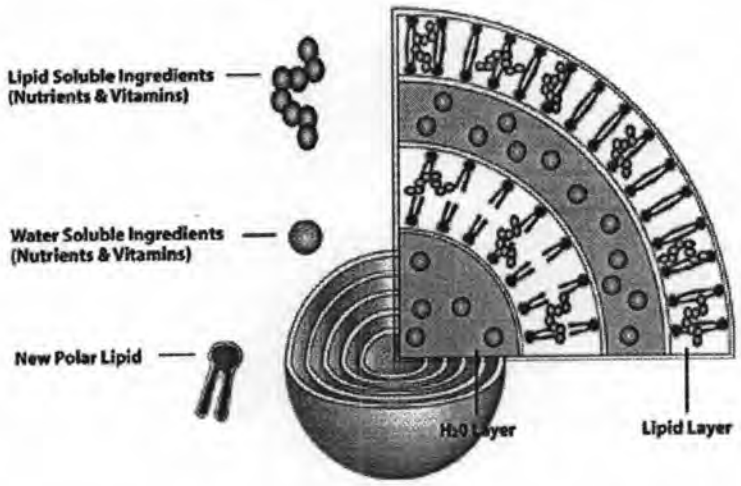

Figure 3 Structure of liposomes

Liposomes are simply vesicles in which an aqueous volume is entirely enclosed by a membrane composed of lipid molecules (usually phospholipids). They form spontaneously when these lipids are dispersed in aqueous media, giving rise to a population of vesicles which may range in size from tens of nanometers to tens of microns in diameter. They can be constructed so that they entrap quantities of materials both within their aqueous compartment and within the membrane(Figure 3). The value of liposomes as model membrane systems derives from the fact that liposomes can be constructed of natural constituents such that the liposome membrane forms a bilayer structure which is in principal identical to the lipid portion of natural cell membranes. The similarity between liposomes and natural membranes can be increased by extensive chemical modification of the liposomal membrane, and may be exploited in areas such as drug targeting or immune modulation, both in vivo and in vitro (Roger, 1989).

The simplest concept of preparation in mechanical dispersion is drying the lipid composition down onto a round bottom flask and then dispersed by addition of the aqueous medium, followed by shaking(Figure 4). Even before exposure to water,

the lipids in the dried-down form are thought to be oriented in such a way as to separate hydrophilic and hydrophobic regions from each other, in a manner not unlike their conformation in the finished membrane preparation. Upon hydration, the lipids are said to swell and peel off the support in sheets, generally to form multilamellar vesicles. In this method, the aqueous volume enclosed within the lipid membrane is usually only a small proportion of the total volume used for compounds to be entrapped, although the absolute yield of material may be satisfactory for practical purposes. Lipid soluble compounds on the other hand, can be encapsulated to 100% efficiency, providing they are not present in quantities which overwhelm the structural components of the membrane.

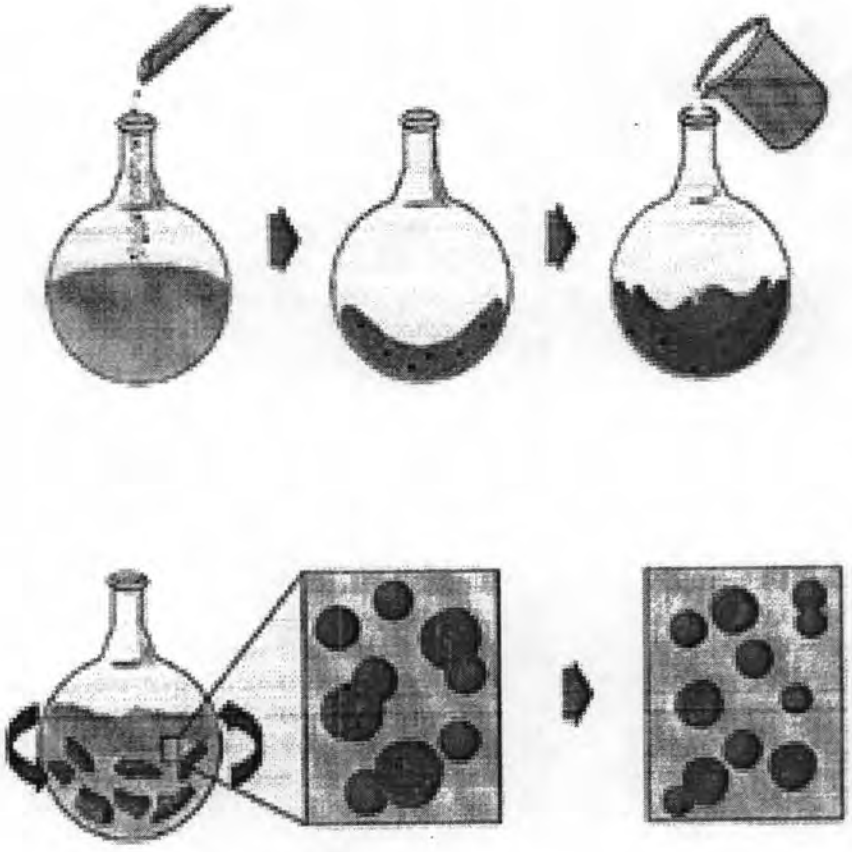

Figure 4 The preparation of liposomes

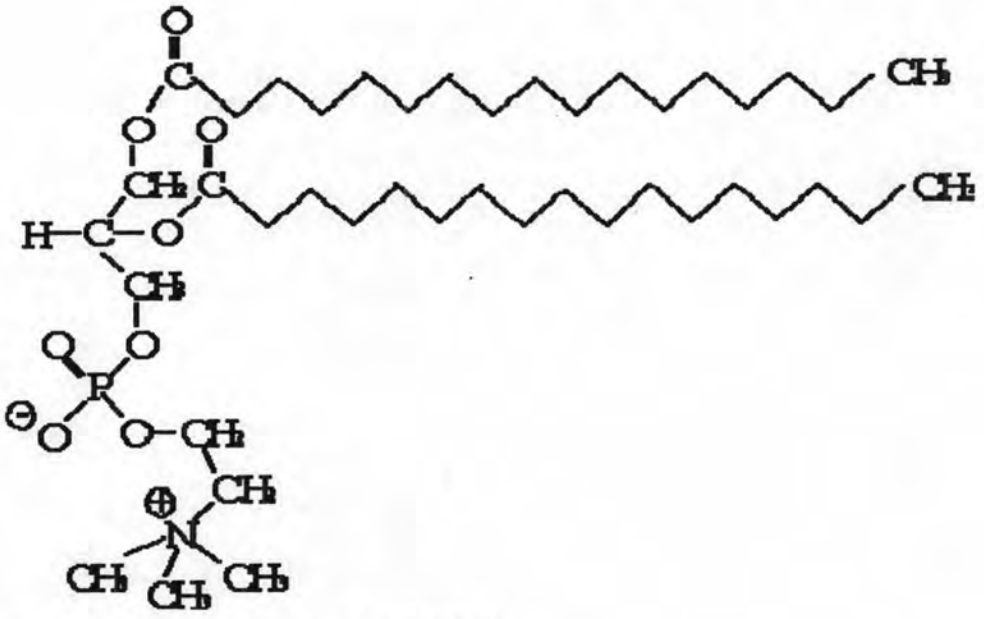

Figure 5 Structure of phosphatidylcholine

Phospholipids are the major structural components of biological membranes. The most common phospholipids are phosphatidylcholine (PC) molecules which have amphipathic molecules in which a glycerol bridge links a pair of hydrophobic acyl hydrocarbon chains, with a hydrophilic polar head group, phosphocholine(Figure 5). Molecules of PC are not soluble in water. Phophatidylcholines contrast markedly with other amphipathic molecules (detergents, lysolecithin) in that bilayer sheets are formed in preference to micellar structures. This is thought to be because the double fatty acid chain gives the molecule an overall tubular shape, more suitable for aggregation in planar sheets compared with detergents with a polar head and single chain, whose conical shape fits nicely into aspherical micellar structure (Roger, 1989).

Phosphatidylcholine, also form known as lecithin can be derived from both natural and synthetic sources. They are readily extracted from egg yolk and soy bean but less readily from bovine heart and spinal cord. In this study, Phosphatidylcholine is extracted from egg yolk. Forming as they do the major phophoipid component of many cell membranes, they are often used as the principal phospholipid in liposomes for a wide range of applications, both because of their low cost relative to other phospholipids, and their neutral charge and chemical inertness, Lecithin from natural sources is in fact a mixture of phosphtidylcholines, each with chains of different length and varying degrees of unsaturation. Lecithin from plant sources has a high

level of polyunsaturation in the fatty acyl chains, while that from mammalian sources contains a higher proportion of fully saturated chains (Roger, 1989).

Phase transitions at different temperatures, lecithin membranes can exist in different phases, and transitions from one phase to another can be detected by physical techniques at the temperature is increased. The most consistently observed of these phase transitions is the one occurring at the highest temperature, in which the membrane passes from a tightly ordered gel or solid phase, to a liquid-crystal phase at raised temperatures where the freedom of movement of individual molecules is higher. In general increasing the chain length, or increasing the saturation of the chains increases the transition temperature, membranes made from egg yolk lecithin have a transition temperature from -15°C to -6°C compared with membranes from mammalian sources which are usually in the range zero to 40°C.

An understanding of phase transitions and fluidity of phospholipids membranes is important both in the manufacture and exploitation of liposomes since the phase behavior of a liposome membrane determines such properties as permeability, fusion, aggregation, and protein binding, all of which can markedly affect the stability of liposomes, and their behavior in biological systems (Roger, 1989).

Sterols are important components of most natural membranes, and incorporation of sterols into liposome bilayers can bring about major changes in the properties of these membranes.

Cholesterol (Figure 6) does not by itself form bilayer structures, but it can be incorporated into phospholipids membranes in very high concentrations up to 1:1 or even 2:1 molar ratios of cholesterol to PC. In natural membranes, the molar ratio varies from 0.1-1 depending upon the anatomical and cellular location. Being an amphipathic molecule, cholesterol inserts into the membrane with its hydroxyl group oriented towards the aqueous surface and the aliphatic chain aligned parallel to the acyl chains in the centre of the bilayer. The 3β–hydroxyl group is positioned level with the carboxyl residues of the ester linkages in the phospholipids, with very little

vertical freedom of movement. The presence of the rigid steroid nucleus alongside the first ten or so carbons of the phospholipids chain has the effect of reducing the freedom of motion of these carbons, while at the same time crating space for a wide range of movement for the remaining carbons towards the terminal end of the chain above a certain concentration of cholesterol, the membrane area occupied by the acyl chains and sterol combined is greater than or equal to that taken up by the phosphocholine head group, so that PC membranes with high levels of cholesterol do not show the chain tile that is observed in the gel phase of liposomes composed of pure PC. Addition of cholesterol to PC membranes has a marginal effect on the position of the main transition temperature (T_c) in DPPC the T_c changes from 41°C to 44°C with 33 mol% cholesterol. With increasing concentration, however, cholesterol is able to eliminate evidence of a phase transition altogether, reducing the enthalpy of phase change to zero at 50 mol% (1:1 ratio) and in so doing altering the fluidity of the membrane both below and above the phase transition temperature, below this temperature the phospholipids are pushed apart, the packing of the head groups is weakened and the fluidity of the ordered gel phase is increased. Above the transition temperature, the reduction in freedom of acyl chains causes the membranes to condense with a reduction in area closer packing and a decrease in fluidity. These changes in fluidity are paralleled by changes in permeability of the membrane-decreased by high cholesterol at temperatures higher than the T_c but increased at lower temperatures (Roger, 1989).

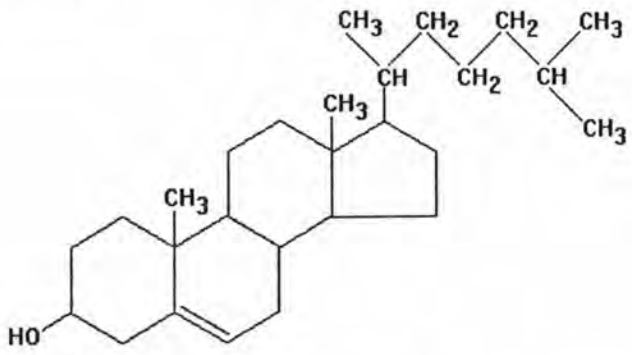

Figure 6 Structure of Choresterol

Cholesterol has an effect similar to alpha-tocopherol on membrane fluidity. Alpha-tocopherol and cholesterol has independent effects in increasing the polarization of DPH (1,6-diphenyl-1,3,5-hexatriene) above the phase-transition

temperature when the two compounds are incorporated together into liposomes(Fukuzawa, et al., 1980).

6. Cholesterol ester

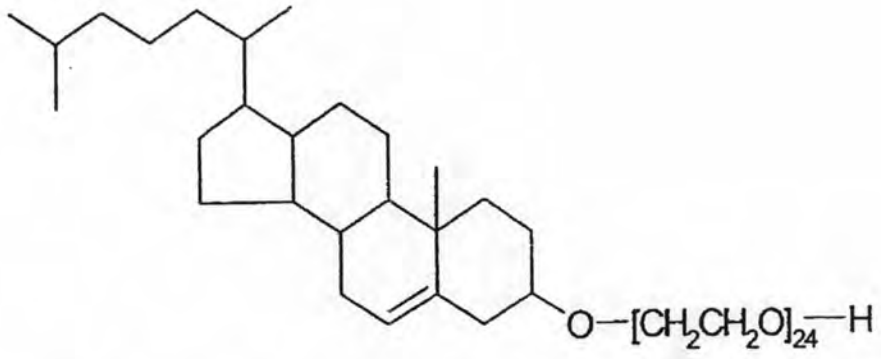

Figure 7 Structure of cholesterol ester

Many non-ionic surfactants are used to stabilize liposomes with difference in toxicity. The ester bond in the alkyl ester surfactant can be hydrolyzed in the body and therefore, this kind of surfactant hasless toxicity than alkyl ester surfactants. For example, sorbitan monostearate (a alkyl ester surfactant) contanin 10% by mole of solulan C-24, is not haemotoxic (Uchegbu and Vyas, 1998). Moreover, the sorbitan ester surfactants are commonly used as pharmaceutical excipients. A hydrophilic lipophilic balance (HLB) is a good indicator for vesicle forming ability. A HLB value of between 4 and 8 was found to be suitable for vesicle formation (Yoshioka, 1994 and Uchegbu, 1995).

7. Stability of liposomes

There are many changes can take place in liposomes when storage during the time. The phopholipid can occur chemical degradation-oxidation and hydrolysis. Liposomes maintained in aqueous suspension may aggregate, fuse or leak their contents.

Prevention of chemical degradation can be kept to a minimum of oxidation by start with freshly purified lipids and freshly distilled solvents, avoid procedures which

involve high temperature, carry out the manufacture process in the absence of oxygen by deoxygenating aqueous solution with nitrogen, store all liposome suspensions in an inert atmosphere and include an anti-oxidant as a component of the lipid membrane

Hydrolysis of the ester linkages will proceed most slowly at pH values close to neutral. However, even at low pH, such as that required for active loadin of drugs, hydrolysis can be kept to a minimum if scrupulous attention is paid to the removal of residual solvent from the dried lipids.

Physical stability of mean particle and particle size distribution by Mastersizer is based on the principle of laser ensemble light scattering. The instrument is composed of lens 300 RF counting the size in the rage of 0.05-3480 μm. The light from a 2-mW Helium-Neon laser (633 nm wavelength is used to form an analyzer beam. The transmitter and receivers units mounted on an optical bed. The Fourier lens mount receiver and any particles introduced by the sample presentation modules present within it will scatter this laser light.

One of the simplest theories used is the Fraunhofer model. This model can predict the scattering pattern that is created when a solid, opaque disc, of a known size is passed through a laser beam. This model is satisfactory for some particles but it does not describe the scattering exactly. Very few particles are disc shaped and most particles are transparent. The accepted theory which accurately predicts the light scattering behaviour of all materials under all conditions is known as the Mie theory. Mie theory was developed to predict the way light is scattered by spherical particles and deals with the way light passes through, or is adsorbed by, the particle. This theory is more accurate, but it does assume that you know some specific information about your particle, such as its refractive index and its absorption. The key point about these theories is that if you know the size of the particle and other details about its structure, you can accurately predict the way it will scatter light. Each size of particle will have its own characteristic scattering pattern, like a fingerprint, that is unlike any other size of particle. The Mastersizer works backward from the above theories by using the optical unit to capture the actual sattering pattern from a field of particles, then, using the theories described above, it calculate the size of particles that created the pattern.

The detector array within the optical unit is made up of many individual detectors. Each detector array within the optical unit is made up of many individual detectors. Each detector will collect the light scattering from a particular range of angles. The detector array takes a snap-shot of the scattering pattern. Obviously this snap-shot will only capture the scattering pattern from the particles that are passing through the analyzer beam at that particular time. Taking only one snap-shot may not give the representative reading of the scattering pattern. To overcome this, the Mastersizer takes many snap-shots (known as snaps) and averages the result. Typically over 2000 snaps are made for each measurement, with each snap taking 1ms.