Manufacture of liposomes: A review

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A brief introduction to liposomes and the methods of their preparation is presented. Various laboratory-scale and large-scale methods of preparation of liposomes are reviewed. They include some of the recent techniques like the 'bubble' method, microencapsulation, microfluidization, LLC method, ethanol injection, freon injection and detergent dialysis and the traditional methods like hydration, sonication and reverse phase evaporation. This paper also discusses the crucial problems like stability and quality control in the commercialization of liposomes and the methods (such as lyophilization and the various characterization techniques like electron microscopy and gel permeation chromatography) to overcome them. Also discussed is the mechanism of vesicle formation.

LIPOSOME research is gaining importance in biological, pharmaceutical and medical research because liposomes seem to be the most effective carriers for the introduction of all kinds of agents into cells. Liposomes, which are lipid microspheres, have a wide spectrum of applications ranging from use in basic research in biophysics to various practical applications like cosmetics and pharmaceuticals, production of ultrafine particles, and many more. There are several key references, including books and review papers, published recently which deal with the topic of liposomes exhaustively[1–10].

Liposomes are used as model systems for studying biomembranes and their properties, such as permeability, as a function of chemical composition of the membrane[11, 12]. In biochemistry, they enable the scientists to reconstitute and investigate membrane proteins in a well-defined environment which closely mimics the natural one[13–15]. The most important use of liposomes is expected to be in pharmacology, medicine and biotechnology, where they serve as vehicles for controlling the delivery of entrapped drugs, genetic material, enzymes and other macromolecules[16, 17]. Liposomes have also attracted the attention of the food and cosmetic industries, where it is possible to form insoluble molecules in water-based liposomes, pastes and ointments[18]. Several large pharmaceutical companies like Ciba-Geigy, Upjohn, Beckton Dickinson, Squibb, along with specialized companies like Liposome Technology, Vestar and The Liposome Company, are active in manufacturing liposome-based products. The pharmacology department of KEM Hospital, Bombay, through its liposome research centre under Prof. Kshirsagar is conducting human trials of certain liposome-entrapped drugs[19]. The other Indian laboratories active in liposome research include Liposome Research Centre, University of Delhi, IIT Kanpur, Hamdard University, Delhi, Indian Institute of Chemical Biology, Calcutta, Indian Toxicology Research Centre, Lucknow, School of Life Sciences, Nehru University, Delhi, Department of Pharmacological Sciences, Panjab University and TIFR, Bombay. Research in these laboratoriés has been centred on the pharmacological applications and the chemistry of lipids[20–31]. In the coming years one sees an enormous potential for liposome manufacturing as more and more industrial manufacturing methods are developed. Products currently under investigation in various companies include immunomodulators, cancer chemotherapeutics and diagnostics, antibiotics, ophthalmics, antiasthmatics, antifungals, vaccines and gastric protectants[32, 33]. Liposomes are also used as a support for semiconductor particles in applications such as the photoconversion of solar energy[34].

The most important commercial product to date is the liposomal formulation agent Amphotericin B, which has a greatly improved therapeutic index and has been successfully employed for treatment of systemic fungal infection in cancer and organ transplant patients[35].

Morphology of liposomes

The Greek root of the word liposome means, a 'fat body', but one may define it as a hollow structure made up of phospholipids having a multilayered structure (Figure 1). The lipid molecule consists of a polar, hydrophilic 'head' generally consisting of a phosphate group. The tail consists of two long hydrocarbon chains. To minimize the unfavourable interactions, lipid molecules are arranged in the form of a bilayer wherein the heads form the surfaces of a sandwich protecting the tails from interacting with water. To avoid the ends of the fat bilayered sheet being exposed to water, they wrap up to form closed spherical structures[36]. The thickness of the membrane is around 4–5 nm.

According to the definition provided by the New York Academy of Sciences[37], liposomes are classified as follows:

1. Multilamellar large vesicles — MLV 0.1–6 μm.
2. Small unilamellar vesicles — SUV 0.02–0.05 μm.
3. Large unilamellar vesicles — LUV > 0.06 μm.

Some of the other shapes and structures encountered are oligolamellar[38], giant unilamellar[39], multivesicular[40], stable pausilamellar[41], helical[42] and cochleate[43].

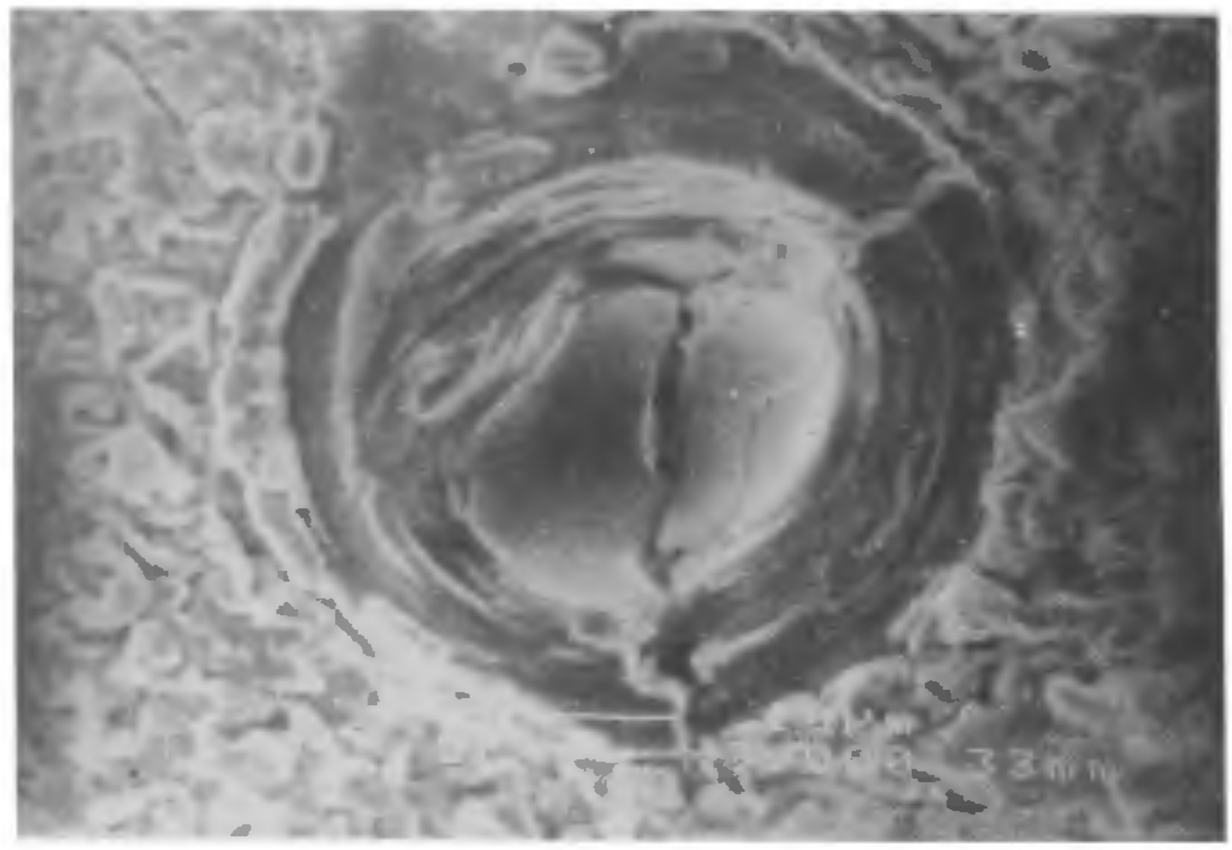

Figure 1. The internal structure of a liposome directly imaged by cryoscanning electron microscopy of a fractured specimen.

Sometimes, especially in the case of technological applications, the term 'liposome' is used for SUV, LUV and MLV, while in the older literature MLV and liposome are used synonymously and vesicles usually indicate unilamellar structures.

The procedures for liposome preparation can be generalized and divided into three stages[10]:

1. Preparation of the aqueous and lipid phases.
2. Primary processing involving lipid hydration.
3. Secondary processing steps (essential in some cases and optional in others).

Recently, one-step preparation of liposomes has gained popularity, especially for the industrial-scale preparation of liposomes. The chemical composition of the lipid bilayer, the size distribution of the liposomes and the number of layers in each liposome are the key parameters according to which one can classify liposomes. The size of liposomes is also important in pharmacokinetics[44, 45].

An ideal method of preparation should be simple, standardized, reproducible and cost-effective and the yield should be homogeneous and stable for sufficient periods of time. A liposome preparation technique must provide reproducible preparation of extremely homogeneous unilamellar liposomes on a micro–macro scale. Moreover, the size of the liposome should be controllable over a large range. This is particularly important in commercial applications of liposomes.

Scope

The primary aim of this paper is to discuss potential commercial and commercial manufacturing techniques of liposomes. It also provides a synoptic account of manufacture-related issues like methods to achieve stability and analytical methods for quality control. A brief description of the mechanism of vesicle formation is also included.

Outline

This paper is outlined as follows. The next section deals with the mechanism of vesicle formation. Then some of the various surfactants that have been used for liposome production are listed. This is followed by a description of the various traditional methods of preparation of liposomes along with some of the recent commercial techniques used for large-scale manufacture. Post-treatment needed for 'Stealth' liposomes is also mentioned. Then an account is given of the various issues (like stability and sterilization) which are important from the industrial point of view. Also the analytical methods used for the characterization of liposomes are mentioned. The last section lists the conclusions, and gives the scope for further research.

Mechanism of vesicle formation

Though many methods of preparation of liposomes have been reported, there is a dearth of literature enunciating the mechanism of vesicle formation. When water is added to the dry phospholipid film, the outer monolayer hydrates more than the inner ones. Blisters are formed due to the increased surface area of the polar heads with increasing hydration. Water penetrates in between the bilayers as well as through the bumps. The hydration reaction reduces the energy of the system, which causes the system to increase its specific surface area. The polar heads are exposed to water maximally. The distance between two lamellae is a compromise between the

repulsive steric and the attractive van der Waals forces. Upon agitation these tubes detach immediately, sealing off their exposed edges and form MLVs. The intensity of agitation influences the size of the MLVs formed[46].

Recently, Lasic[47] has proposed that all liposomes form from existing bilayers in two ways. A small section of a flat bilayer may break off and close upon itself or part of the bilayer may bud off from the large aggregate if it is forced to curve. It is possible to force a curve into the bilayer by introducing agents that increase the area of the outer lipid monolayer relative to the area of the inner layer. This can be achieved by increasing the size of the polar heads of the outer lipids. For example, ionizing the outer monolayer attracts water molecules that bind to the polar heads, effectively increasing their size. The area of the outer monolayer can also be increased by intercalating certain amphiphilic molecules such as detergents, among the existing lipids[47].

Other models include one by Szoka and Papahadjopoulos[48] which explains the preparation of liposomes by reverse phase evaporation[48]. Finer *et al.*[49] have proposed a mechanism of vesicle formation by sonication. It is based on rearrangement of bilayer fragments into vesicles. Evans[50] proposes that vesicles form by budding off of highly curved sections of larger lipid sheets. Vesicle formation has also been described by other authors[51–54]. The main analytical techniques used by these workers include electron microscopy and light scattering experiments.

Surfactants used for preparation of liposomes

Liposomes have been prepared by using various types of lipids, lipid mixtures and other amphiphiles. The lipid structure, lipid bilayer, and bilayer interactions have been reviewed by Cevc[55]. From the viewpoint of liposome design phosphatidylcholine (PC) derived from hen egg yolk is the most popular choice due to its availability in large quantities, high purity and bioadaptability[56, 57]. A compilation of other phospholipids which are used in the production of liposomes can be found in the review by Szoka and Papahadjopoulos[58] and the original references therein. Cholesterol is added to the lipid in various ratios, primarily to decrease the permeability of phospholipid bilayers and to improve the stability[59, 60], although some authors have proposed that cholesterol has no effect on liposome stability[61, 62]. Instead, cholesterol used may be autoxidized over time, leading to loss of the encapsulated material[63, 64].

The net surface charge of liposomes can be modified by addition of compounds such as phosphatidyl serine (PS) or phosphatidyl glycerol (PG) for negatively charged liposomes. Negative charge has many advantages such as increase in efficiency of the uptake of liposomes by cells *in vitro*[65] and long $t_{1/2}$ in blood[66]. The phase transition temperature (T_c) of the fatty acyl groups of phospholipids is also a key parameter. At temperatures above T_c, liposomes are designated as 'fluid', whereas below this temperature they are 'solid'[67]. In forming liposomes it is essential[59] that hydration of dried lipid is done at a temperature higher than T_c.

Liposomes are also made from synthetic lipids. These lipids have polymerizable groups in their fatty acid chains, or polar heads or both, and are completely stable even under extreme physical conditions[68–71]. Amphiphiles such as didodecyl dimethyl ammonium bromide[72], polyoxyethylene[46] and hydrogenated castor oil ether[73] have also been investigated. The use of synthetic lipids is severely limited by its very high cost[74].

Laboratory methods of preparation

Multilamellar vesicles

Hydration of dry lipid films is the classic method of preparation (Figure 2). In fact, this pioneering experiment performed in Cambridge, England, introduced the phospholipid vesicles[75, 76]. It involves shaking a dry phospholipid film in water or buffer, the film having been previously deposited on the wall of a round-bottom glass flask by evaporation from organic phase. To achieve better results, hydration is followed by extrusion or sonication.

Entrapment of aqueous solutes by MLV is improved when the lipid is dried in the presence of the aqueous solute to be entrapped, thus forming a mixed lipid film with the solute trapped between layers. This mixed film is then hydrated gradually with a minimum of aqueous phase. Preformed liposomes are added to an aqueous solution of the solute and the mixture is either lyophilized or evaporated. Subsequent rehydration forms MLVs[77–79]. Repeated freezing followed by thawing is a convenient method for increasing the trapped volume of MLV[80, 81].

Small unilamellar vesicles

Sonication is the principal method of preparation in vogue[82–84] (Figure 3). Sonication of MLVs or other aqueous dispersions of phospholipids produces SUVs

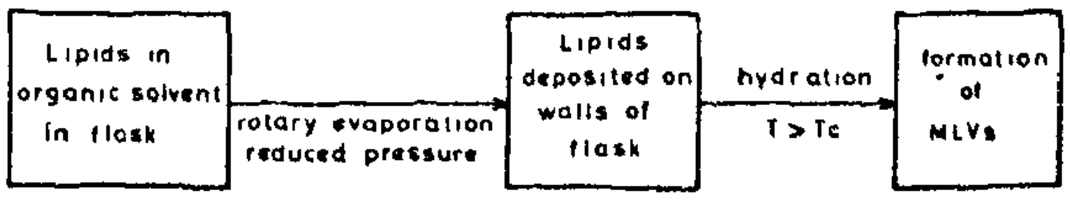

Figure 2. Hydration Steps in the preparation of liposomes – a schematic view. The lipids are deposited from organic solvents on the wall of a round bottom flask, followed by addition of aqueous buffer to produce MLVs

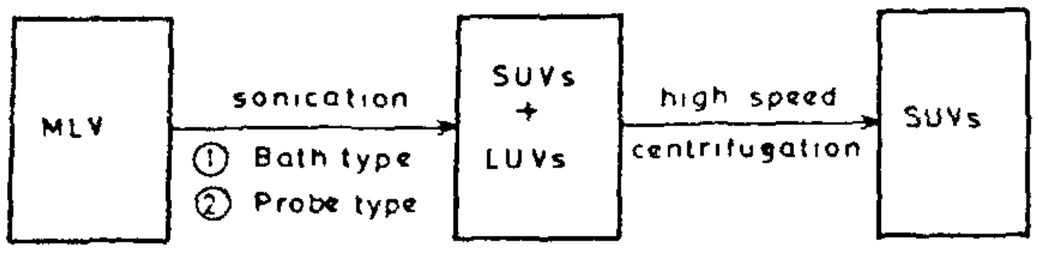

Figure 3. Sonication The popular method of producing liposomes, in which energy is added to MLV dispersion in the form of sound waves to break it into SUVs.

entrapping an aqueous space. High-speed centrifugation is a simple process to separate larger vesicles.

Although sonication with a probe usually can deliver a high power density, and hence faster breakdown of the MLV to structures with a minimal radius, it has the disadvantage of contaminating the preparation with metal from the tip of the probe, leading to degradation of the phospholipid. Moreover, this technique can generate aerosols from solutions containing radioactive traces, carcinogenic chemicals or infectious agents that have been added to the preparations, which can be a serious biohazard[58]. Hence, bath-type sonicators are preferred. They require longer times and reproducibility is hard to achieve[85].

Homogeneous preparations of SUV can be obtained by the use of a power laboratory press (the French press). In this apparatus, dispersions can be subjected to high hydraulic pressure[86]. Homogenization of MLVs or other lipid dispersions produce SUVs. The principal consideration is the shear force generated, temperature of operation and lipid fluidity at that temperature[87, 88]. Recently, preparation of liposomes using a Mini-Lab 8.30 H high-pressure homogenizer has been reported[89]. Injection of lipid solutions into a solvent miscible with the aqueous phase is a simple method for preparing SUVs. However, it is limited by the need for subsequent processing to remove the solvent, possibility of residual solvent, and the low solubility of some lipid components in aqueous-phase-miscible solvents such as ethanol[90] (Figure 4). SUVs can also be formed spontaneously during hydration of certain lipids, such as a mixture of short- and long-chain lecithins[47]. SUVs can be formed by swelling of slightly charged phospholipid films deposited on special supports in excess water[91].

Large unilamellar vesicles

Either dried lipid mixtures or preformed liposomes are solubilized with the detergent-containing aqueous phase. Detergent removal from the mixed micelles leads to LUVs[92]. Detergent dialysis or gel permeation column can be used for detergent removal.

Reverse phase evaporation produces LUVs. In this method, the drug-containing aqueous phase is emulsified in the presence of phospholipids in diethyl ether (Figure 5). By evaporation of the solvent under vacuum, LUVs are formed[48, 93].

Injection of solutions of lipid in volatile solvents into an aqueous phase under reduced pressure results in hydration of lipids producing LUVs[94]. A procedure is suggested which involves dissolving the lipid in an organic solvent, washing with a mild acid, removing the solvent, adding salt solution and adjusting the pH (raising pH to 10 and lowering it immediately to 7.55)[95, 96]. In this method, MLVs are reorganized to LUVs taking advantage of lipid hydration and structural changes following deprotonation of acidic lipids.

Calcium addition to appropriate SUV induces fusion and results in cochleate cylinders. Addition of EDTA produces LUVs[97]. Spontaneous vesiculation[98] also leads to formation of LUVs. Liposomes are also produced by using microfabricated structures[99].

Angelova[100, 101] has described a method based on the effect of a.c. electric fields on the lipid swelling and liposome formation. The basic phenomenon is sequences of lateral fusions of SUVs induced by the applied a.c. electric field.

A new physicochemical method for preparing liposomes, called the lamellar liquid crystalline (LLC) method, has recently been developed[102]. Liposomes appear spontaneously at the interface between water and an LLC phase containing a large amount of propylene glycol, glycerol and water. Lamellar liquid crystalline phase consists of thick stacks of multiple lipid bilayers[103]. These break into smaller fragments closing upon themselves, thus entrapping some solvent in the interior, forming liposomes[104].

LUVs can be formed by extruding MLV dispersions through polycarbonate membranes with defined diameter pores, resulting in a decrease in particle size and polydispersity[105, 106].

'Stealth' liposomes

It has been found that a majority of the injected liposomes are cleared from the blood system by phagocytic cells

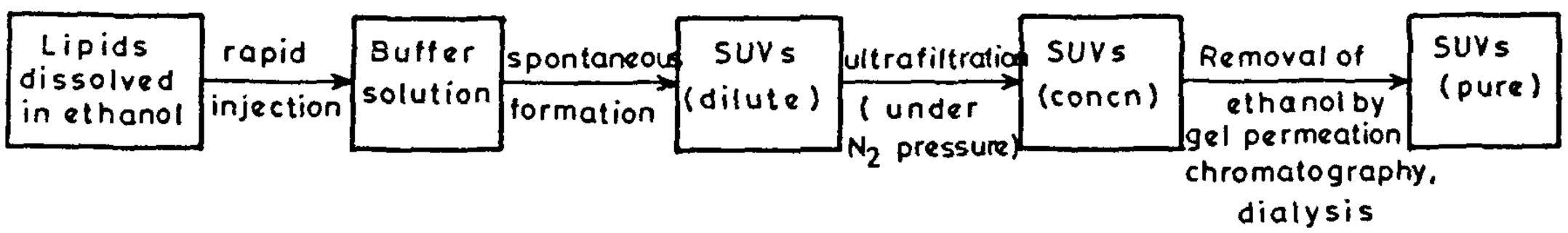

Figure 4. Ethanol injection: Lipids dissolved in ethanol are rapidly injected into an aqueous solution to form SUVs.

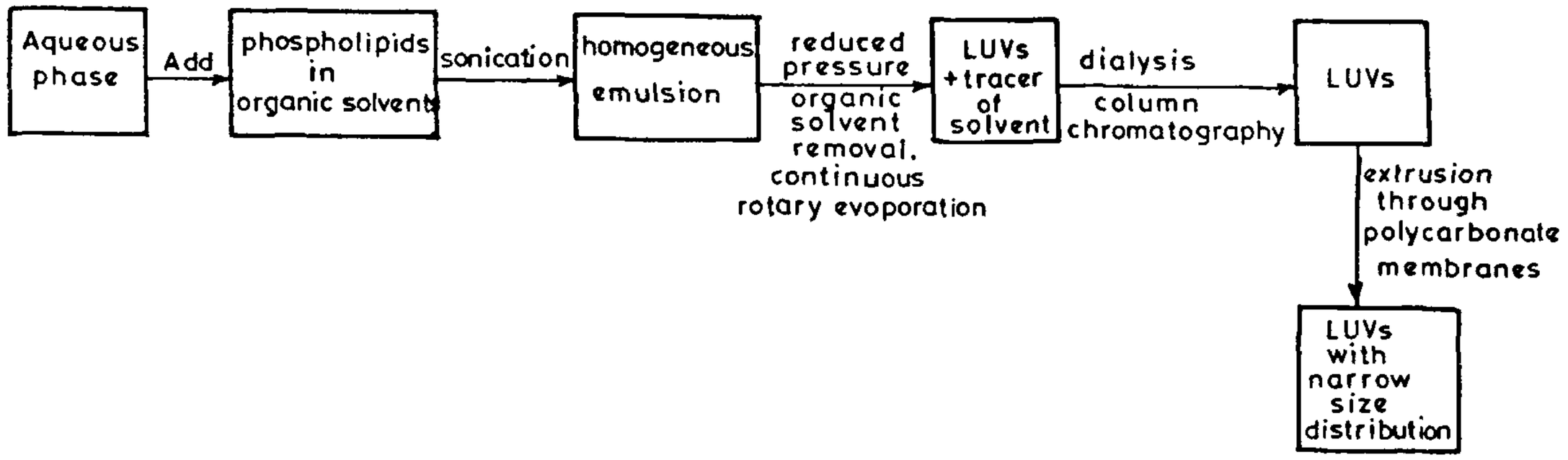

Figure 5. Reverse phase evaporation. LUVs are formed from water-in-oil emulsions of phospholipids, followed by evaporation of solvents from the oil phase.

of the reticuloendothelial system (RES) such as macrophages. Therefore, to target the non-RES tissues, stealth liposomes are used[107]. These were developed by Allen[108] of the University of Alberta, Canada, and by Gabizon[66] at Liposome Technology, Inc. These liposomes have a protective carbohydrate coating that mimics the surface of red blood cells to avoid detection and uptake by RES. Prolonged blood circulation can be achieved by use of polysialic acids[109]. A method is described for ^{67}Ga-labelling liposomes containing a polyethylene glycol coating exhibiting prolonged blood circulation[110].

Commercial methods of preparation

The methods described below are the recent industrial-scale manufacturing methods for liposomes. Some of these methods are currently used in pharmaceutical industry, like Freon injection, microfluidization, ethanol injection and detergent dialysis. The microencapsulation technique and the 'bubble' method are under development.

Freon injection method

It is a promising industrial method[111]. A solution of lipids in Freon is injected into an aqueous solution at the same rate as Freon is being evaporated. Hydrophilic molecules to be encapsulated are dissolved in the aqueous phase while the hydrophobic ones are coinjected with the solution. This being a continuous process is easy to scale up.

Detergent dialysis technique

Recently, Dianorm, Germany, has introduced a new unit process[112] for the production of liposomes called LIPOSOMAT (Figure 6). The continuous detergent removal from the equilibrated mixed lipid/detergent micelle solution leads spontaneously to the formation of liposomes. The detergents used are gentle in their action and do not hydrolyse or peroxidize liposome contents, e.g. sodium chlorate and *n*-octyl-glycoside. The flat dialysis membranes are prepared from natural cellulose. The detergent removal is performed at a constant temperature which is above the transition temperature of the lipid mixture. Continued dialysis leads to a practically detergent-free liposome preparation. The production capacity of LIPOSOMAT plant can be up to thousands of litres per day. The size of the liposomes depends on dialysis rate, type of detergent, type of lipid(s), lipid/detergent molar ratio, lipid concentration, electrolyte content and pH[113].

Microfluidization

Microfluidization consists of processing emulsions under high pressures through an apparatus called a microfluidizer[114] (Figure 7). The main advantage of microfluidization is continuous production of large quantities of lipid vesicles without dissolving the phospholipids in organic solvents[87, 115, 116].

The other advantages in using microfluidization for liposome production are: (i) the evaporation step can be eliminated; (ii) it is possible to handle high lipid concentrations and consequently to obtain high encapsulation efficiencies; (iii) the mean diameter of the liposomes can be controlled by selecting the pressure and the number of cycles[117]. The usual problems associated with extrusion, like clogging, are circumvented[87].

The apparatus essentially consists of an interaction chamber in which the pressurized MLV stream is directed into precisely defined microchannels ($2 \times 100\,\mu m$). The stream is then accelerated to a laminar velocity of about 100 m/s and then these separate streams are made to collide, resulting in turbulence and cavitation forces and formation of liposomes[118].

Microencapsulation techniques

It is also called the double emulsion technique and was reported by Battele Memorial Institute, US[119]. Matsomoto and others have also reported this method[120]. The conditions for the preparation of lipid vesicles by the in-water drying method, one of the microencapsulation techniques, is described in detail by Ishii and others[121] (*Figure 8*). This process consists of the following steps: (i) dispersion of an aqueous solution of the drug into an organic solvent to yield a water-in-oil (w/o) emulsion. (ii) dispersion of this w/o emulsion into an aqueous phase; (iii) formation of a lipid membrane on the surface of droplets by evaporation of the organic solvent from the oil phase; and (iv) removal of the free drug. The size of liposomes and encapsulation efficiency is reported to be dependent on the rpm of the stirrer used to form emulsions. Stability, over long time, is also reported.

Bubble method

Talsma and others[122] have developed a new production technique for one-step preparation of liposomes that looks very promising for large-scale production of liposomes containing vulnerable substances like proteins which are sensitive to high shear forces or incompatible with organic solvents and detergents. This method is based on the introduction of large quantities of gas bubbles into a lipid dispersion. During bubbling, homogeneous vesicle dispersion is formed.

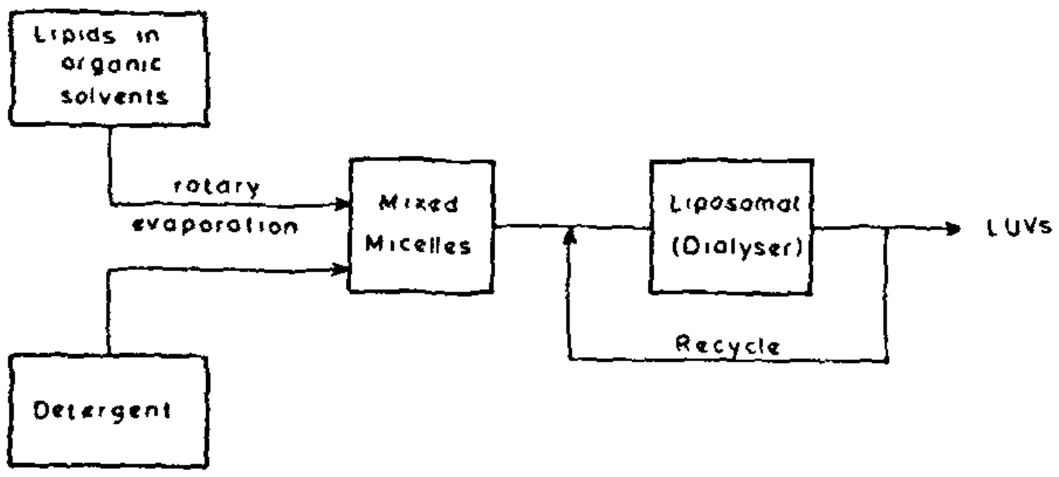

Figure 6. Detergent depletion: LUVs are formed by a continuous process consisting of detergent solubilization and removal.

Ethanol injection technique

Recently, the development of the first approved liposome dermatic has been reported[123]. This method is similar to the solvent injection method described earlier and does not depend on hazardous solvents or detergents. The size, encapsulation efficiency, lamellarity and stability are well-controlled and reproducible.

Industrial applicability

For the successful employment of liposomes as drug delivery systems, stability of the liposome product has to be assured. Since liposome-encapsulated drugs will be used as human and veterinary pharmaceuticals, the final formulations must be stable for eighteen months to two years. For the drugs sold over the counter they should be stable at least at room temperature. The causes of chemical and physical instability and the stabilization procedures are reviewed by Ausborn *et al.*[124].

Autoxidation and hydrolysis are the two main processes which degrade the phospholipids. Autoxidation generates toxic products while hydrolysis greatly increases the permeability of liposomes[125, 126]. Chemical stabilization of liposome dispersions is mainly achieved by modifying the bilayer mixture. Antioxidants such as α-tocopherol are added or fully hydrogenated phospholipids are used.

Liposomes have a large content of free energy due to their extreme curvature. They have a natural tendency to fuse[127]. This results in the loss of encapsulated drug. Manipulation of the bilayer composition and cholesterol has resulted in partially improved stability. Freezing and lyophilization are the two commonly used methods for stabilization of liposomes. To achieve stability and active targetability of liposomes *in vitro* and *in vivo*, a methodology involving coating the outermost surface of liposomes with a naturally occurring polysaccharide that bears a cholesterol moiety as a hydrophobic anchor to penetrate the liposomal bilayer is proposed[128]. Lyophilization is a process of drying in which water is sublimed from the product after it is frozen. As the process is carried out under vacuum, degradation by oxidation is prevented and due to the low temperatures adverse chemical effects

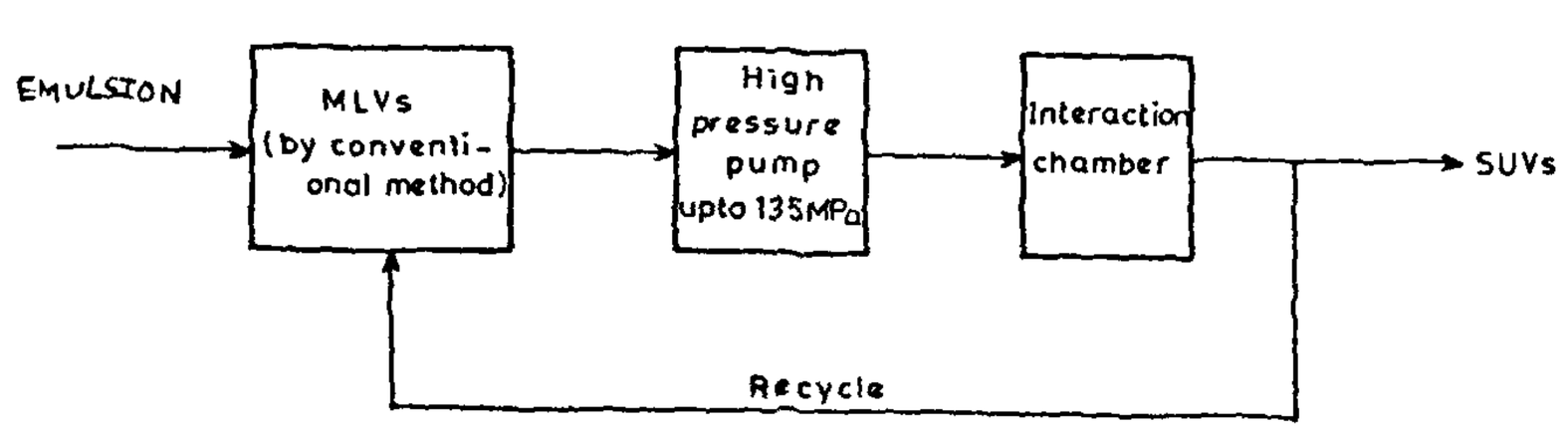

Figure 7. Microfluidization. SUVs are manufactured in a continuous system in a microfluidizer, wherein there is a collision of two high-pressure feed streams.

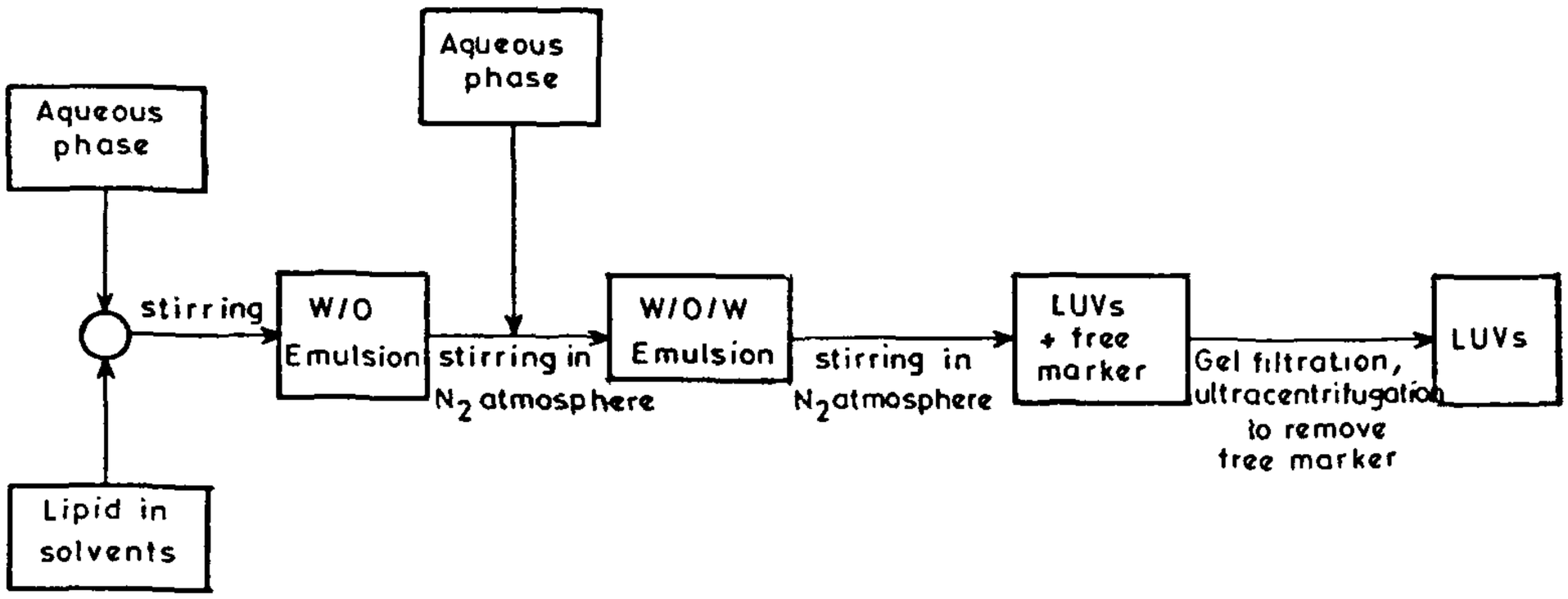

Figure 8. Microencapsulation: LUVs are produced by the in-water drying method without the use of detergents or sonication.

Table 1. Comparison of traditional methods of preparation of liposomes

Method	Diameter (nm)	Captured volume (l/mol lipid)	Efficiency of encapsulation (% of original volume)	Whether homogeneous yield?	Scale-up possibility reported?	Features
Hydration method	400–3500	4	5–15	No	No	Easy, quick preparation, low trapped volume, relative stability on storage
Sonication	20–50	0 2–1 5	0.1–1.0	Yes	No	Contamination with metal, nonuniform sonication, fast procedure
Reverse phase evaporation	100–1000	7–11	30–50	No	No	High trapping efficiency, technically complex, exposure to solvents
Detergent removal	200–1000	2–7	12–22	Yes	Yes	Exposure to detergents, lengthy procedure, reproducible yield
Ethanol removal	30–120	0.4–1.5	0.5	No	Yes	Fast, simple, low encapsulation, concentration step required
Freeze thawing	250–500	2–5	31–89	No	Yes	Solute-dependent, fast procedure

do not take place[129, 130]. Also, lyophilized preparations would not require special storage conditions as in freeze–thaw processes[131]. In both processes, the role of cryoprotectants is important. The mechanism of cryoprotection due to saccharides is reviewed by Miyajima and Tanaka[132]. Analytical methods used by them include Raman and NMR spectroscopy and DSC. They conclude that the liposome lyophilized with a disaccharide showed the strongest stability during the rehydration process.

The use of membrane-bound cryoprotectants has been suggested in place of conventional cryoprotectants[133]. Efficient drying methods allow the production of liposome powders which upon rehydration do not lose integrity[134]. Methods have been devised to produce liposomes with an electric potential across the membrane[135, 136]. When some charged drugs are added to these liposomes, they are pulled through the membrane into the aqueous space with greater than 95% efficiency. This implies that drug can be introduced just before the treatment.

Other issues which are important from the industrial point of view such as reproducibility of the preparation, pyrogen content, integrity of the lipid, patentability, sterility, cost, toxicity and quality control methods are briefly discussed by Ostro[74]. Ostro suggests that sterile processing is the only possible method of sterilization though it is time-consuming and expensive. Other methods of sterilization popular in the pharmaceutical industry, like terminal heat sterilization and γ-ray treatment, cannot be used as they irreparably damage the liposomes, though recently sterilization of liposomes, under proper conditions, without or with thermostable, lipophilic drugs by autoclaving has been reported[137]. Use of HPLC is suggested to determine the total quantity and the quality of drug in the final formulation[138, 139]. Quasielastic light scattering and Coulter counter can be used for determination of the size distribution. Particle size distribution affects the effectiveness of the liposome formulation[140]. Hence, it is an important parameter.

Presently, many techniques like electron microscopy[141, 142], gel permeation chromatography[143, 144], dynamic light scattering[145, 146] and light microscopy are in vogue. The choice of method depends on the size of liposomes[147]. Except for electron microscopy, no other method has the capability of resolving the distribution over the entire range, from 10 nm to 50 μm. Moreover, microscopy gives images directly and, hence, is model-independent. For routine commercial use, electron microscopy is limited by its cost. Turbidity has also been used to characterize the average size but is limited by many other factors that also affect turbidity[148]. Ultracentrifugation[149] and nuclear magnetic resonance (NMR)[150] are also used to obtain the distribution of small homogeneous vesicle populations.

Conclusions

1. Drug carrier systems presently under investigation include antibodies, glycoproteins, cells, reconstituted viruses and liposomes. Among the various other candidates proposed, liposomes stand out as the most promising candidate, not only because they are biodegradable and easy to prepare but also because they are versatile in terms of composition, size and other structural characteristics.
2. Important parameters defining a liposome are: lipid : cholesterol ratio, size of liposome, lamellarity, transition temperature and any coating like that of polysaccharides required for stability.
3. Regarding the mechanism of vesicle formation, the explanation generally revolves around the spontaneity of the process, though some progress in understanding the mechanism has recently been made. Very short life of the intermediate structures severely limits the study of formation mechanism.
4. As the manufacturing methods are being developed, in most of the cases no optimization in relation to parameters like energy, cost and stability is reported. The commercial process details are not available as these are patented.
5. For designing new methods one can borrow ideas from other similar fields like emulsion preparation.

Liposome research is a continually progressing field. It needs a proper co-ordination of various disciplines like biochemistry, biophysics, cell biology, medicine and engineering sciences. A lot more work is needed in identifying new raw materials for vesicle preparation, for which it will be necessary to conduct experiments on various lipids. Also, much is desired for liposomes to become pharmaceutically acceptable products, especially regarding stability, sterility and quality control. Appropriate apparatus like homogenizers needs to be developed for liposome preparation. This will result in liposomes being widely used in products of everyday life, rather than being an object of academic curiosity only.

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