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Review

Liposomes as sterile preparations and limitations of sterilisation techniques in liposomal manufacturing

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ABSTRACT

Liposomes have been widely researched as a delivery system and there have been many manufacturing techniques used in the production of liposomal preparations, the most common being extrusion method which will be introduced in this paper. However because of the unique properties of liposomes and their susceptibility to chemical and physical degradation, sterilisation remains an unresolved issue in the manufacturing of liposome-based formulations. It is especially pertinent in the pharmaceutical industry where liposomes are commonly prepared for intravenous administration. Currently, filtration and aseptic manufacturing are recommended for the preparation of sterile liposomal products. Newer aseptic manufacturing techniques such as dense gas techniques have been devised to eliminate the need for terminal sterilisation. This paper will highlight the limitations of the conventional techniques that are specific to the liposome preparation under the respective sterilisation conditions specified by the 2011 British Pharmacopoeia to achieve 10^{-6} Sterility Assurance Level, as well as modifications incorporated in the newer sterilisation technologies to overcome these limitations. This paper will introduce these techniques in brief, including their advantages and limitations.

1. Introduction

Liposomes are concentric lipid bilayer vesicles in which an aqueous volume is encapsulated. They are commonly formulated from phospholipids and sterols and may contain hydrophilic polymer conjugated lipids. These ingredients can be derived from natural sources such as egg, soy or milk which can contain contaminants such as endotoxins, therefore product sterilisation is required, especially when the end product is designated for formulation of parenteral (mainly intravenous) products. The composition of the preparation will determine its physicochemical properties such as phase transition temperature and these in turn determine its suitability towards the various sterilisation techniques.

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Due to their chemical composition, liposomes are biodegradable delivery systems that can be used to solubilise substances with low aqueous solubility such as lipophilic drugs which, if injected as a free drug, might precipitate in aqueous blood media and contribute to the formation of a thrombus. The entrapped drugs consequently are protected from physiological degradation and released in a sustain-release manner, enhancing their bioavailability and circulation time. The therapeutic index of cytotoxic drugs such as anticancer drugs can also be raised when their liposomal carriers are affixed with cell-specific antibodies or ligands. In view of their advantages as delivery systems, liposomes are increasingly being researched and utilised in the pharmaceutical, food and cosmetic industries. In the pharmaceutical industry, liposomes are designed as carriers to deliver bioactive agents into cells, immunological adjuvants and more recently, contrast agents for molecular imaging [1].

In spite of all the advantages, much research is still required before liposomes can be extensively utilised in pharmaceutical formulations due to issues of stability, reproducibility, entrapment efficiency, size distribution, short circulation half-life of vesicles and more importantly, sterilisation [2]. The following sections of this paper will summarise some of the degradation mechanisms of liposomes and how these mechanisms will be induced during the conventional sterilisation techniques (at conditions specified by the 2011 British Pharmacopoeia), therefore making these techniques unsuitable, to a certain extent, in the manufacturing of liposomes.

2. Common manufacturing techniques of liposomal products

Liposomes of varying sizes and lamellarity are formed spontaneously when lipid components are introduced into an aqueous environment. Due to the hydrophobic force, the amphiphilic constituent molecules cluster into aggregates in order to minimise the contact between their hydrophobic portions and the surrounding aqueous environment, these aggregates can be organised into liposomes if provided with adequate amount of energy in the form of heating, sonication or homogenisation. However, for liposomal preparations to be acceptable as pharmaceuticals, these liposomes must be processed to fulfil criteria such as a defined size and narrow size distribution hence various manufacturing techniques have been developed to achieve the uniformity of the vesicles.

One of the most common manufacturing techniques in industries is the extrusion method. Liposomes are prepared prior to the extrusion procedure by a simple process of dissolving the lipid component in an organic solvent before emulsifying this mixture in an aqueous phase and slowly evaporating the organic solvent. The liposomes formed are then, in their liquid-crystalline state, extruded through polycarbonate filters with defined pore sizes using pressurised N_2 gas at temperatures above the phase transition temperature [3]. The production of a sterile preparation using this method is possible if pore sizes of 200 nm are used. However, for most of the other manufacturing techniques, terminal sterilisation is still required and the susceptibility of the liposomes to various chemical and physical degradation mechanisms has rendered conventional sterilisation techniques unsuitable. Some of the main degradation pathways include oxidation, hydrolysis, phase transition and aggregation. In the following sections, the various modes of liposomal degradation will be explained before the discussion of the advantages and disadvantages of conventional, as well as a novel sterilisation strategy of liposomal formulations.

2.1. *Lipid peroxidation*

Lipid peroxidation is the oxidative degradation of lipids and liposomes, in particular, are easily oxidised even in absence of specific oxidants. It has been reported that radicals, such as O_2^- and $OH^\bullet$ (often resulting from the ionisation of water due to irradiation) are involved in the lipid peroxidation of lipid bilayer membranes, especially for those containing cholesterol and phospholipids with a high content of polyunsaturated fatty acid chains [1].

Both radicals can interact with the unsaturated fatty acids and initiate the free radical chain reaction which leads to lipid peroxidation as illustrated in Fig. 1. Alternatively, the superoxide radical can damage the liposomal membrane by generating $OH^\bullet$ and O_2^- which will directly oxidise the unsaturated fatty acids [4]. The hydroxyl radical can also damage the membrane directly by incorporating itself into the double bonds of the unsaturated fatty acid chains [5]. Lipid peroxidation involves the formation of conjugated dienes and malondialdehyde and results in membrane modifications such as permeability, rigidity and conformational changes which can reduce the shelf-life of the liposomes [6].

2.2. *Liposomal hydrolysis*

Research has shown that hydrolysis of both saturated and unsaturated phospholipids are mainly catalysed by OH^- and H^+, with OH^- catalysed hydrolysis being more dominant than H^+ catalysed hydrolysis [7]. In the presence of water, phospholipids can hydrolyse into fatty acids and lysophospholipids which are further hydrolysed into glycerophospho-compounds and fatty acids.

The products of hydrolysis, namely lysophospholipids can increase the membrane permeability of the liposomes significantly, even at low concentrations. The incorporation of the resultant fatty acids in the liposomal membranes will promote fusion of the liposomes which will lead to leakage of contents [8]. The fatty acids can also influence the phase transition temperature and hence stability of the liposomal preparation in a pH-dependent manner. This will be discussed in Section 2.3.

Rate of hydrolysis is influenced by pH, temperature and buffer concentration [9]. Fortunately, studies by Mustafa *et al* have shown that hydrolytic rate is relatively low at physiological pH hence the parenteral liposomes will not be subjected to hydrolysis significantly when injected into the bloodstream. Similarly, hydrolytic rate is found to be independent of ionic strength hence addition of tonicity adjusting agent NaCl in parenteral liposome formulation should not predispose the preparation to hydrolytic degradation. However, the same studies conducted show that addition of buffer (e.g. acetate and citrate), a crucial component of parenteral

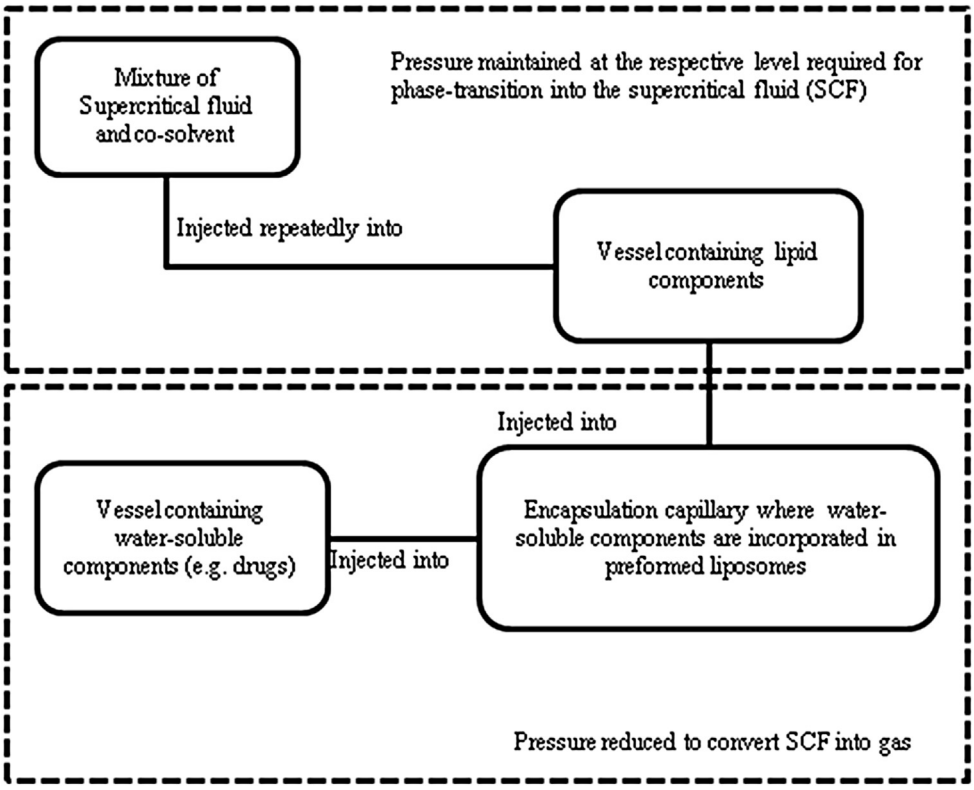

Fig. 1 – Schematic representation of the key procedures involved in dense gas technique.

preparations, can increase the hydrolytic rate of the liposomal preparation. The same studies have also concluded that an increase in temperature can directly lead to an increase in the hydrolytic rate.

2.3. *Phase transition*

Phase transition of liposomes occurs when the physical state of the lipid bilayer changes from an ordered gel conformation (with fully extended hydrocarbon chains) to a disordered liquid-crystalline state (randomly oriented hydrocarbon chains). Factors that promote phase transition of the lipid bilayer membrane include high temperature, as well as factors that can lower the phase transition temperature (T_c) of the phospholipids [10]. Transition of the lipid bilayer membranes from gel to liquid-crystalline state is governed by T_c, which is the temperature at which the ordered packing of the lipid bilayer membrane will be lost and the membrane fluidity will increase substantially. Factors influencing T_c include pH, concentration of free fatty acids and cholesterols in the bilayers. The main significance of phase transition is that the flexibility and permeability of the lipid bilayer membrane will increase when it undergoes phase transition, causing it to become transiently leaky and the encapsulated materials to be released.

At low pH, the incorporated fatty acids relieve the crowding of phospholipid polar groups, permitting the phospholipid acyl chains to pack more closely together hence T_c increases. At high pH, the free fatty acids are deprotonated and this will generate a negative charge density in the bilayer surface which results in a repulsive effect between the phospholipid acyl chains that lowers the T_c [10]. Other factors contributing to a lower T_c include an increase in free fatty acid concentration and a decrease in cholesterol concentration, both of which can result from certain sterilisation procedures which will be discussed later.

2.4. *Aggregation*

Liposomes are thermodynamically unstable colloidal system and they tend to aggregate over time and when the Van der Waals attractive (V_A) forces are stronger than the electrical double layer repulsive forces (V_R) that exist between approaching vesicles, as dictated by the theory of colloidal stability. Factors that can affect the degree of aggregation include temperature, ionic strength and surface charge of the liposomes which can be quantified using zeta potential (ζ). It is postulated that at high temperatures, the heat energy of the lipid bilayer membrane is sufficient to overcome the potential barrier of aggregation V_{max}, hence the colloidal system tends towards aggregation [11].

A major problem from aggregation of liposomes is the destabilisation of the lipid bilayer membrane and the subsequent release of entrapped content [11]. Aggregation of the liposomes will also lead to fusion and precipitation of the vesicles, hence product destabilisation. Unlike other conventional parenteral products, liposomal formulations cannot incorporate dispersing agents, a common formulation additive in parenteral preparations to prevent aggregation, because these agents will insert themselves into the lipid bilayer membrane and induce formation of pores, causing

content leakage at sublytic concentrations and lysing the vesicles at higher concentrations [12].

3. Conventional sterilisation techniques

Bearing in mind the unique composition and properties of liposomes hence their susceptibility to the aforementioned degradation mechanisms, it is important to note that conditions required in conventional sterilisation techniques (all except filtration) can be detrimental to the stability of the liposomal preparations. To discuss the feasibility of conventional sterilisation techniques in liposomal preparation, Table 1 has been included as a summary on the various limitations and advantages of the conventional sterilisation techniques in the manufacturing of conventional sterile dosage forms, with emphasis on the more recent dosage form of liposomes.

3.1. *Filtration*

This method of sterilisation involves the filtering of liposome preparations through sterile filtration units under pressure. Screen filters made from polycarbonate or depth filters made from cellulose acetate can be used to retain particles that are larger than 200 nm, allowing liposomes smaller than 200 nm to pass through. This sterilisation technique is suitable for thermolabile products, which include liposomes, since it does not involve any form of heating nor conditions that can result in the formation of degradation products or leakage of liposomal contents associated with the other terminal sterilisation techniques. One drawback of this technique is that filtration must be performed under aseptic conditions and is a relatively expensive method since it requires equipment to work under high pressure, which could be above 25 kg/cm^2 [13].

Some may contend that the size restriction limits the applicability of this terminal sterilisation technique. However, this limitation is insignificant in manufacturing liposomes for parenteral usage since a small vesicle size (of <600 nm, with an average of 300 nm) is recommended to minimise complications such as retention and trapping of the vesicles in the narrower capillaries (e.g. in the lungs). For preparations of size range between 200 and 300 nm, the formulations can be heated above the phase transition temperature so that they can pass through the filter pores in their less rigid spherical conformations [14].

Filtration sterilisation is relatively time-consuming and not efficient for removal of viruses [15]. The choice of tight filter holders and filtration units are essential to the sterility of the preparations, since the external pressure exerted on the liposomal dispersion might displace the filter holders slightly causing the assembly to be leaky. Studies have shown that polycarbonate membranes are less effective in ensuring the sterility of the preparations, as compared to Milex® and Minisart® filtration units [14]. The limitations of this technique have prompted research of the other sterilisation techniques. Unfortunately, all the other conventional techniques result in the formation of degradation products via the aforementioned degradation pathways.

3.2. *Gamma (γ) irradiation*

This sterilisation technique achieves microbial death primarily through the degradation of microbial DNA and disruption of the microbial membranes via free radical formation. Although γ-irradiation is an effective sterilisation method for certain medicaments and surgical equipment, it cannot be used in liposome sterilisation. By the aforementioned mechanism of free radical formation, the unsaturated phospholipids of liposomes are subjected to peroxidation and destabilised under irradiation at the recommended dose

Table 1 – Summary of the advantages and limitations of the techniques used in sterilisation of liposomes.

Sterilisation technique	Advantage(s)	Limitation(s)	Relative cost	Convenience
Filtration	Low operating temperature	Aseptic conditions are required; Only applicable to liposomal system below 200 nm in diameter; High pressure (25 kg/cm^2 and above) is required	High	Low
γ-irradiation	Highest reliability in killing; moderate operating temperature (35–40°); High penetration into products	Operates only on a large scale; conditions might result in degradation of liposomes	High	High
Saturated steam	Cost and convenience	Conditions might result in degradation of liposomes	Low	High
Dry heat	Cost and convenience; depyrogenation can be achieved	Conditions might result in degradation of liposomes	Low	High
Ethylene oxide	Low operating temperature	Carcinogenic residues might remain	Moderate	Low
UV sterilisation	Cost and convenience	Poor penetration into products; Conditions might result in degradation of liposomes	Low	High
Dense gas technique	Fast, single step processing, moderate operating temperature; Minimal or no use of organic solvent; Good solvent properties of supercritical fluid	Elevated pressure is required	High	High

of 25 kGy. More specifically, γ-irradiation of cholesterol produces OH•, while irradiation of phospholipids produces O_2^- [5].

In addition to lipid peroxidation, irradiation-induced liposomal degradation is also attributed to the free radical phospholipid fragmentation [16]. Acidic degradation products resultant of the fragmentation process include phosphatidic acid and free fatty acids fragmented from the unsaturated phospholipids (mainly phosphatidylglycerol) can contribute to a pH reduction in the liposomal preparation [17]. Compared to other parenteral preparations, liposomes are especially susceptible to irradiation-induced degradation because of the synergism between the two degradation pathways. It has also been shown that the presence of fragmentation products can promote lipid peroxidation [16].

While several studies have shown that oxidation can be minimised by adding antioxidants (e.g. chelating agents) which are common additives of conventional parenteral formulations, and using an inert and light-resistant storage atmosphere which helps to enhance the antioxidants' effectiveness, these measures do not suffice in eradicating the extensive lipid peroxidation sustained from the γ-irradiation. Related studies have shown that reducing agents such as conventional antioxidants and trehalose sugars can disrupt the radical formation process. The use of chelating agents can inactivate the transition metals in the liposomal preparations from mediating the radical formation process. More recent studies have shown that removal of water via freeze-drying can reduce the formation of OH• hence reducing the irradiation-induced liposomal damage [18]. Freeze-drying is also a preferential method for preparing lyophilised liposomes because these are thermolabile products and the conditions used in freeze-drying do not involve heat.

The removal of oxygen, a common practice adopted to minimise irradiation-induced damage in parenteral formulations, is ineffective in liposomal preparation because the fragmentation mechanism is promoted in absence of oxygen [16]. Fortunately, γ-irradiation might still be applicable for the sterilisation of liposomes containing solid saturated phospholipids [19]. In solid preparations, the absence of water which is a major source of the hydroxyl radicals, will suppress the oxidative degradation induced by the irradiation process [18].

Oxidative degradation can be reduced by freezing or freeze-drying liposomes prior to irradiation (a common technique used in conventional parenteral product formulation to minimise undesired effects of γ-irradiation), since radical movement is reduced significantly in the frozen or lyophilised liposomes. However, it is important to note that irradiation-induced degradation of the lyophilised phospholipids might still occur due to presence of residual water which serves as the source of free radicals. It has been shown that when liposomes are irradiated in a frozen state, there is no apparent phospholipid and cholesterol degradation given that water molecules are a main source of free radicals [20]. Using another analytical method, another research group has reported irradiation-induced degradation of solid/frozen/lyophilised saturated phospholipids and attributed this to the presence of residual water in the lyophilisates which renders the lyophilisates prone to indirect radical attack [17].

3.3. *Saturated steam sterilisation*

Unlike γ-irradiation, saturated steam sterilisation does not pose problems of lipid peroxidation since it occurs in absence of air, thus oxygen, preventing free radical formation. It is hypothesised that the high sterilisation temperature has reduced the concentration of dissolved oxygen in the liposomal dispersion insofar as oxidative degradation is inhibited [21] and that peroxidation will occur under autoclaving conditions because it is a temperature-dependent process [13].

Cholesterol hydrolysis is also an insignificant contributor to liposomal degradation in this sterilisation technique. Instead, terminal steam sterilisation of liposomal preparations (15–20 min at 121 °C) is faced with problems common to conventional parenteral emulsions, namely hydrolytic degradation of phospholipids and vesicle aggregation; conventional parenteral emulsions are observed to undergo hydrolysis and cracking when autoclaved. An additional problem unique to liposomes is the resultant leakage of encapsulated contents [22].

Under this sterilisation technique, condensed steam transfers large amount of heat to the liposomal preparations in order to kill the microorganisms. However, a side effect of this heat transfer is the acceleration of the hydrolysis in the preparations given that hydrolytic rate is directly influenced by temperature. The increase in temperature also promotes aggregation in accordance with the explanations provided in Section 2.4. Finally, content leakage will result from the hydrolysis- and aggregation-induced fusion of the liposomes. The increased membrane permeability following the phase transition of the membrane at the autoclaving temperature (which is usually above T_c) also contributes to the content leakage [21].

However, it has been shown that terminal steam sterilisation can still be a viable option in liposome formulation processes by limiting the hydrolytic degradation with the proper choice of buffer composition and pH [21,22]. It is also concluded that rate constant of hydrolysis is lowest at pH 6.5 and decreases with lower buffer concentration [7]. Hydrolysis of the phospholipids follows Arrhenius kinetics, whereby rate constant increases with temperature. It is concluded that autoclaving at neutral pH does not result in significant hydrolytic degradation whereas substantial hydrolytic degradation is observed at pH 4 [21]. Aggregation of liposomes depends on the liposomal dispersion used (i.e. type, charge of phospholipids and buffer composition); hence, it can be minimised with the right composition of liposomal dispersion.

3.4. *Dry heat sterilisation*

Dry heat sterilisation is generally unsuitable for manufacturing liposome-based formulations because these formulations are usually prepared in aqueous forms which will evaporate and become unstable when subjected to the high temperature and long exposure time of dry heat sterilisation. The extreme heat will destabilise the formulations by promoting phase transition. A primary mechanism of action for dry heat involves oxidation, which is unfortunately, one main degradation pathway for the unsaturated lipids found in liposomes.

3.5. *Ethylene oxide sterilisation*

Ethylene oxide, being an electrophile, will react with the strongly nucleophilic endocyclic nitrogen atoms in DNA commonly via a Substitution Nucleophilic Bimolecular (S_N2) mechanism (Ehrenberg *et al*, 1981). Ionisation of the NH_2 groups on the nucleic acid molecule (in the presence of water) produces N^- that function as the nucleophile attacking the carbon on ethylene oxide, giving rise to an alkylated molecule.

Unlike the saturated steam and dry heat sterilisation techniques, this chemical 'cold' sterilisation technique utilising ethylene oxide vapours can be used in sterilisation of liposomes which are heat-sensitive preparations. Another advantage is that this technique does not alter the vesicle size [23]. Ethylene oxide is an alkylising agent for bacterial proteins and genetic material hence can serve as an effective wide-spectrum anti-bacterial; it is also effective against viruses. Unlike the other techniques, this does not engender any of the degradation mechanisms mentioned earlier.

The use of ethylene oxide in terminal sterilisation of parenteral formulations must be carried out with caution because residues from an incomplete removal of ethylene oxide vapours and other chemical agents can still pose serious problems of flammability, carcinogenicity and mutagenicity [21]. Therefore, parenteral preparations (including liposomes) that are sterilised using ethylene oxide must be degassed thoroughly after sterilisation [23]. It is important to note that this method is not applicable for aqueous liposomal preparations and mainly for lyophilised liposomes which can be easily reconstituted using water for injection [18].

3.6. *Ultraviolet (UV) sterilisation*

UV sterilisation has limited use in the sterilisation of parenteral formulations because UV light, unlike γ-rays, has poor penetration power and is used mainly for surface sterilisation. Besides, UV sterilisation is not applicable for liposomal preparations because its conditions induce substantial liposomal degradation. It is observed that UV induces free radical formation, causing lipid peroxidation and subsequently, increased membrane permeability [24]. The formation of the free radicals has been attributed to processes such as one-electron redox reactions, high-energy radiation and photolysis, and thermal homolysis of the bonds [24]. Similar to γ-irradiation, rate of free radical formation is dependent on the degree of unsaturation of the liposomal preparations [25].

4. Aseptic manufacturing

Besides terminal sterilisation, aseptic manufacturing processes can facilitate the formulation of sterile parenteral products including liposomes. Raw materials (including organic and aqueous solvents, the natural sources of lipid components as well as other additives such as buffers) are sterilised after passing 200 nm filters [26]. The equipment can be autoclaved and sterilised. Subsequently, the liposomes are prepared via procedures mentioned in Section 2 and then assembled into their containers via aseptic filling. During aseptic filling, the other potential sources of contamination (which include the environmental air, operating personnel and the water for drainage) are critically controlled by performing the filling process on work stations that have been designed to protect the previously sterilised component and equipment. These work stations are, in turn, located in clean rooms (Class-100 environments) which have been designed to facilitate and maintain thorough sterility during the entire operation of aseptic manufacturing.

Unlike terminal sterilisation which is an active process of destroying the contaminants within the finished product, aseptic filling, and aseptic manufacturing, is essentially a passive process of avoiding further contamination of the final preparation. Therefore, there is still a risk of contamination in aseptic manufacturing especially if the initial raw materials are not sterilised adequately. In the aseptic manufacturing of liposomes, the natural sources of lipid components can only be subjected to filtration due to physicochemical degradation and other considerations mentioned earlier in the discussions of the various sterilisation techniques. Since the filter membranes can only retain the particles larger than 200 nm, the final formulation will be contaminated with the viruses present in the initial ingredients. Likewise for all parenteral formulations made from components that cannot be terminally sterilised by non-filtration means, the contaminants residing in the ingredients or introduced during the manufacturing processes cannot be removed from the final product even when aseptic manufacturing is performed.

Unlike terminal sterilisation, the degree of sterility assurance cannot be assessed for aseptic manufacture. In view of this and the possibility of contamination, current regulatory thinking (particularly the FDA) regards aseptic manufacture as a process of last resort. However, the problem of contamination during aseptic filling can be circumvented by incorporating ultrafiltration at the end of the manufacturing process [6]. As for the contaminants (including viruses) initially present in the ingredients that cannot be filtered away, the dense gas technique which is a more recently adopted method of preparing liposomes from lipid and aqueous components can perhaps be introduced at the beginning of the manufacturing process.

5. Dense gas technique

Dense gas technique is a newer method of manufacturing liposomes that eliminates the need for terminal sterilisation. In the supercritical liposome method described by [27], instead of dissolving the lipid components in an organic solvent which can be a potential source of microbial contamination, a supercritical fluid such as CO_2 is used for dissolution of the lipid components. This supercritical fluid possesses solvent properties parallel to that of liquids hence it is able to dissolve the lipid components with ease [15]. Similar to the process mentioned previously under Section 2, the mixture is then combined with the aqueous component and the supercritical solvent is evaporated after pressure reductions [28] as illustrated in Fig. 1 below.

CO_2 is utilised in dense gas processing because it presents advantages of non-flammability, non-toxicity (overcoming the limitation of the chemical 'cold' sterilisation technique),

low cost, and relatively low pressure and temperature (200–300 bar and 60 °C) required for phase transition. More importantly, the antimicrobial properties of CO_2 eliminate the need for terminal sterilisation [1,29]. Due to its low viscosity and elevated diffusion rate, the supercritical CO_2 can pass through the microbial cell walls and membranes easily and dissolve in water to form H_2CO_3, increasing the acidity of the bacterial cytoplasm up to pH 3–3.2 and inactivating enzymes crucial for cellular metabolism. The carbonic acid can then ionise into CO_3^{2-} and HCO_3^- which contribute to intracellular precipitation of salts, further contributing to microbial death [30]. Supercritical CO_2 has been proven to be effective in inactivating viruses via viral elimination and inactivation [31].

This process is gentle and will not result in formation of degradation products, hence overcoming the limitations found in the autoclaving and γ-irradiation sterilisation techniques. Due to its high density, the supercritical CO_2 has deep penetrating properties; hence, this manufacturing method can achieve thorough sterility [32] through mechanical cell rupture and physiological deactivation [29]. However, supercritical CO_2 is less effective against spores compared to vegetative cells. In fact, the use of supercritical CO_2 is so effective that this technique can achieve Sterility Assurance Levels of 10^{-7}. This advanced formulation method allows the production of liposomes sized up to 1500 nm in diameter, unlike filtration sterilisation which restricts liposomal sizes to 200 nm [28]. However, this advanced technique is incapable of inactivating bacterial spores unless used in combination with peracetic acid as an additive [32]. Peracetic acid degrades to acetic acid and water and thus posing no concern of toxicity. An additional limitation of this technique is that the solid components of the supercritical CO_2 readily clog up the nozzle and other equipment [15].

6. Conclusion

Liposomes have great potential as parenteral drug delivery systems however due to the unique properties and composition of liposomes, their amphiphilicity allows the encapsulation of both lipid-soluble and water-soluble substances. However, sterilisation of liposomal preparations remains an issue, with each technique presenting its own limitations. Although filtration does not cause any degradation, it imposes size restrictions on the final products; saturated steam sterilisation may be cheap and easy but it can cause product degradation, likewise for γ-irradiation. Though chemical 'cold' sterilisation does not affect product integrity, residual sterilants can cause toxicity issues. As for UV sterilisation and dry heat sterilisation, they are completely inappropriate in liposomal manufacturing. While aseptic manufacturing and filtration are the most commonly utilised methods of producing parenteral liposomes, the procedures involved are time-consuming and the equipment is extremely expensive and difficult to maintain. This impedes the scaling-up of parenteral liposomal manufacturing and ascribes a high cost to such preparations. Although a combination of the dense gas technique, aseptic manufacturing and filtration can perhaps be used to produce sterile liposomal formulations, this combinatorial manufacturing process may be too cumbersome and time-consuming. Hence, there remains a need for the development of a widely applicable terminal sterilisation technique, one that is efficient, cost-effective and can maintain the physicochemical stability and encapsulated content of the preparation.

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