STABILITY OF LIPOSOMES

K.SWAROOP KUMAR, S.L.V.V.S.N, 2T. JYOTHI*, 3S. NAGENDAR, 3S.DEVIPRIYA , G.VIJAYA KUMAR and 3A.M.S.SUDHAKAR BABU.

1Department of Pharmacology, Vels University, Chennai-600117, India.
2Department of Pharmaceutics, Vikas Institute of Pharmaceutical Sciences, Rajahmundry. (A.P), India
3Department of Pharmaceutics, A.M.Reddy Memorial College of Pharmacy, Narasaraopet, (A.P), India

ABSTRACT

Liposomes are potent drug delivery systems but their efficacy is altered due to instability of vesicles in the blood and GIT. In GIT Reticuloendothelial system, particularly the Kupffer cells in the liver play a major part in removing liposomes from the circulation. In the blood stream, several blood components such as lipoprotein, albumin and divalent cations such as Ca$^{2+}$ and Mg$^{2+}$ destabilizes the pH-sensitive liposomes. Physical stability of liposomes can be altered by aggregation and fusion which are the main sources of instability. Stability of liposomes can be improved by preventing oxidation, aggregation, fusion and stabilizing pH sensitive liposomes by using antioxidants, electrolytes and apolipoproteins A1 respectively.

KEYWORDS: Liposomes, Drug carrier systems, Stability of liposome’s, Antioxidants

INTRODUCTION

Liposomes are one of the most potent candidates for drug carrier systems. However, the efficacy of liposomes as a drug delivery system has not yet been established. One of the reasons for this is the instability of vesicles, particularly in biological media (i.e., blood, GI tract, etc) [1]. In general, in early stages of development, freshly prepared liposomes are used. However, from a pharmaceutical point of view it is important to demonstrate that liposomes can be stored for a long period of time. Degradation process particularly oxidation & hydrolysis may change the properties of an aqueous liposome dispersion [2]. Oxidation of phospholipids can be minimized by using antioxidants or an inert atmosphere [3]. More problematic for the long term chemical stability of aqueous liposome dispersions is the chemical hydrolysis of ester glycerophospholipids to free fatty acids, lysophospholipids & glycerophospho compounds [4-7]. Formation of substantial amount of these hydrolysis products may lead to an increase in particle size [8], an increase in permeability of liposome bilayers [9] and also various effects [10].

HISTORICAL BACKGROUND OF LIPOSOMES

In December 1932 J.Y.Johnson applied for a British patent for Pharmaceutical Preparations hereafter called “depot” capable of holding any desired dose of medicament but releasing it over any desired space of time only gradually without the slightest determinant to the organism. The formula for this “depot” included lecithin, cholesterol & Water and there is little doubt that the Preparation at least in part consisted of liposomes. Stocckenius in 1959,
states that dried Phospholipids swell to form Multilamellar Structures when exposed to water is referred to as “Myelin Figures”. However A.D Bangham in Cambridge who initially observed that these Structures could entrap a range of ions and release them at various rate, indicating that each lamella consisted of a closed Phospholipids bilayer[11]. The multilamellar Phospholipid vesicles, properly called lyotropic Somatic mesophases , are now universally Known as liposomes.

**PREPARATION AND STABILITY OF LIPOSOMES**

Liposomes were prepared by mixing lipids in an organic solvents chloroform: methanol (1:1) in a round bottom flask. The organic solvent was removed under vacuum by rotary evaporation. The dry lipid film, paste or cake is then hydrated. Upon hydration of lipids, large multilamellar vesicles are typically formed. Their size can be reduced by extrusion, homogenization or sonication. Hydrophilic drugs are normally added in the hydrating medium, while hydrophobic drugs are co dissolved in the organic phase. At the selected storage temperature the pH of the dispersion was measured and adjusted before and after extrusion, if necessary. The liposome dispersion was refrigerated overnight. Subsequently the pH of the dispersion was measured again at the storage temperature and adjusted if necessary[12]. The size of the liposomes ranging from nanometers to several micrometers[13].

During Production of liposomes necessary precaution should be taken. It is important to avoid air oxidation of the fatty acyl groups of the component phospholipids. Lipid peroxidation is an estimator of the oxidative stability, has been described to be an important degradation process of poly unsaturated fatty acids[14, 15]. Most of the enrichment diets are basically composed of polyunsaturated fatty acids and hence are susceptible to peroxides when exposed to high temperature and oxygen[16]. Therefore the oxidation process produces terminal toxic compounds such as aldehydes and ketones.

Both the efficiency of entrapment and the rate of leakage will also vary according to the Physical Characteristic of the drug particularly its octanol - Water partition coefficient. Polar drugs such as cytosine arabinoside are entrapped relatively low efficiencies compared to drugs such as daunomycin. Efficiency of entrapment for those drugs is much higher[17]. The rate of leakage from liposomes is generally higher for non-polar drugs such as actinomycin-D than for Polar drugs such as Methotrexate[18].

Protein containing liposomes were rapidly removed from the circulation mainly by the liver and spleen, with little participation by other tissues[19]. Reticuloendothelial system, particularly the Kupffer cells in the liver, play a major part in removing liposomes from the circulation, although there is also electron microscopic evidence that the hepatic parenchyma cell may be involved in liposome clearance[20]. Clearance rates of injected liposomes depend on both the liposome size and charge, with larger size and negative charge resulting in a more rapid rate of removal from the circulation. In addition, it has been suggested that the nature of liposome charge influences their tissue distribution, possible by affecting liposome interaction with cellular membranes[21, 22]. The uptake of liposome bearing cytotoxic drug to other tissues such as bone marrow, small intestine, kidney and heart may be substantially reduced[23-25].

The globulin fraction of Plasma Proteins can bind strongly to liposome's thus influencing both the rate of cellular uptake and the rate of leakage of entrapped drugs[26, 27]. Liposomes, at least those bearing a positive charge, have been found to activate...
the alternative pathway of human complement [28], and this could conceivably lead to lyses of liposome's invivo[29]. Finally, liposome's are also subject to degradation by the action of plasma phospholipids[30]. Cycle-specific drugs such as actinomycin and Cyclophosphamide, whose antitumour activity may be more dependent on the peak concentration of drug achieved rather than duration of exposure [31].

Physical stability of liposomes can be understood as kind of colloidal stability. Stability of colloidal system can be explained by DLVO theory. According to this theory the stability is predicated on the notion that two independent types of forces govern the interaction between similar colloidal particles: attractive Vander walls forces and repulsive forces[32]. One of the most important aspects of physical changes is particle size and size distribution. These changes in colloidal system occur mainly via two mechanisms: at the molecular level, the mechanism can be asymmetric molecular exchange, where as at the particle level it is mostly aggregation, fusion, co-acccervation or flotation/precipitation. In the case of liposomes, aggregation and fusion are the main sources of instability such processes occur to a significant extent over a long period. Aggregation and sedimentation of neutral liposomes is brought about by Vander Waals interactions, and tend to be more pronounced in large vesicles, in which the greater flatness of the membranes allows greater areas of membrane to come into contact with each other. Although factors such as residual solvents and trace elements can enhance this process. Formation of liposome aggregates is a natural and unavoidable phenomenon for uncharged membranes [33].

The medical application of PH-sensitive liposomes requires that the formulation is stable in the blood stream, but several blood components are potential destabilisers of liposomes, including lipoproteins as high density lipoprotein (HDL) and the complement system[34,35]. Albumin has been reported to destabilize dioleoyl phosphotidyl ethanolamine/oleic acid (DOPA/OA) liposomes due to extraction of OA from the membrane and subsequent disintegration of the liposomes and release of contents[36]. The PH-sensitivity decreased after incubation in plasma. Divalent cations, such as Ca$^{2+}$ and Mg$^{2+}$, are important components of biologic fluids and can destabilize anionic liposomes. These cations have been shown to cause massive aggregation and fusion of phosphatidyl serine vesicles[37] and DOPE/OA liposomes[38]. In both cases it was found that Ca$^{2+}$ is a more effective destabilizer than Mg$^{2+}$. The stability of liposomes in gastrointestinal tract is very important if they are to be used as drug carrier by the oral route. Liposomes should be stable against enzymes found in the GIT, bile salts and gastric acidity. The pancreatic lipase was capable of degrading naturally occurring phospholipids. It has been found that liposomes containing short chain fatty acids were more stable against destructive action of lipase[39].

Cycle specific drugs such as actinomycin D and cyclophosphamide, whose anti tumor activity may be more dependent on the peak concentration of the drug achieved rather than the duration of exposure [40]. In one study in which liposome entrapped actinomycin D was marked reduction in toxicity. This is mainly due to a decrease in its inhibitory effect on intestinal epithelium and on bone marrow stem cells which was related to reduce initial uptake of actinomycin D in these sites [41, 42].

REMEDIES

Liposomes are used as carriers for drugs and diagnostic agents. In general in early stages of development freshly prepared liposome’s are
used, however from a pharmaceutical point of view it is important to demonstrate that liposome’s can be stored for a long period of time. Few precautions are necessary for the satisfactory production of liposomes for drug entrapment.

- To avoid air oxidation of the fatty acyl groups of the component phospholipids, this produces lyso-compounds and free fatty acids which will modify the liposome structure. The usual precaution is to keep stored lipids as well as prepared liposomes in an atmosphere of nitrogen or inert gas such as argon.

- Oxidation of phospholipids is probably not a major problem, since it can be minimized by preventive and protective measures such as the use of antioxidants[^3].

- Instability of liposomes in colloidal system is due to aggregation and fusion at the molecular level. The simplest way to overcome it is to introduce charge into the lipid mixture. Electrostatic repulsions sufficient to stabilize liposome in vitro. As zeta potential is a very good index of the magnitude of the repulsive interaction between colloidal particles. It is commonly used to assess the stability of colloidal sol[^43].

- PH-sensitive liposomal formulations were stabilized by human plasma, probably caused by insertion of apolipoprotein A1 in the membrane[^44, 45] or by exchange of unsaturated lipids with saturated analogues might improve the blood stability of pH-sensitive liposomes.

- To maintain the stability of liposomes against enzymes in GIT, bile salts and gastric acids by replacing natural phospholipids with short chain fatty acids or by adding some cholesterol in the formulation of liposomes[^46].

**CONCLUSION**

In summary it can be stated that the early promise of liposome’s as selective drug carriers remains unfulfilled, as discussed earlier major problems are predominant hepatic and spleen uptake of liposomes, together with destruction of liposomes in the circulation. In addition the capacity of liposomes to enter malignant cells within solid tumours in vivo, and further studies are clearly necessary. If this capability can eventually be demonstrated satisfactorily, the fact that such uptake may quantitatively be much less than that by liver and spleen does not necessarily render the approach invalid, since it is enhancement of tumour cell uptake relative to other target sites such as bone marrow and small intestine which is the main aim of liposome entrapment. In order to maximize on this property, the next step would therefore be a proper appreciation of the modifications in structure necessary to improve on liposome stability in vivo. It is clear that research on tumor cell membrane structure and function continues to make steady progress and in the near future a more sophisticated approach to the design of liposomes for selective delivery to tumor cells should be possible.

**REFERENCES**


22. Richardson VJ, Jewkes RF, Ryman BE, Tattersall MHN, Possible tumour localization of 99m Te-


37. Wilschut J, Duzgunes N, Papahadjopoulos D. Calcium/magnesium specificity in membrane fusion: kinetics of aggregation and fusion of phosphotidylserine vesicles and the role of


