

Liposome Solutions for Poorly Soluble Drugs

Afzal UR-Rahman Mohammed and Yvonne Perrie

Medicines Research Unit, Aston Pharmacy School, Aston University, Birmingham, B4 7ET.



Dr Mohammed studied for an undergraduate degree in Pharmacy (1994-1999) and completed his Masters in molecular sciences (Pharmacology and Biotechnology) at Sheffield Hallam University, UK (1999-2001). In 2001 he completed his PhD investigating liposomes as solubilising agents in Dr Perrie's research group. His current research involves the design of candidate liposomal vaccine delivery systems for the formulation of TB vaccines.



Dr Perrie is a lecturer in Pharmaceutics/Drug Delivery within the Medicines Research Unit, Aston University. Her research is focused on the strategic development of particulate-based delivery systems for the delivery of vaccines, biopharmaceuticals and nucleic acid-based therapies. Prior to her appointment at Aston University, Dr Perrie spent several years developing liposome-based systems in the laboratories of Professor Gregory Gregoriadis at the University of London.

Introduction

Thousands of new compounds can be synthesised each year thanks to developments in combinatorial chemistry and high iv throughput screening. This has led to a dramatic acceleration in drug discovery output. However, this has not come without fresh problems, with many new lead compounds now reflecting the properties of the screening library to a greater extent (Di and Kerns, 2003). Furthermore, the common practice of adopting non-aqueous solvents in the screening process often causes the poor water solubility of compounds to be masked (Curatolo, 1998), resulting in many new entities displaying poor solubility and poor bioavailability below the therapeutic threshold (Kawakami *et al.*, 2002).

Formulation strategies to improve drug solubility: Current problems

In the case of liquid formulations, there are a number of solubility enhancing technologies currently used, including buffers, salts and pH adjustment, co-solvents, cyclodextrins, emulsions, surfactant agents and liposomes. However, several of these current strategies are not without associated problems. For example, paclitaxel, a poorly soluble anti-tumour agent, lacks functional groups which are ionisable within the physiological pH range. Co-solvents are also useful solubilisation tools as they can provide an exponential increase in solubility, in addition to allowing the exclusion of water which can be beneficial for drugs susceptible to hydrolysis. However, limitations to this method include possible drug precipitation after administration, pain, inflammation and haemolysis (Strickley, 2004), with the levels of the tolerated co-solvent being dependent on the route of administration. Alternatively, cyclodextrins can be employed to enhance solubility up to 100 fold (Flynn, 1984). However, their application is still limited by poor drug loading and weak reservoir effect which can result in dissociation of drug-

cyclodextrin complexes upon dilution by plasma and extracellular fluids (Mesens and Putteman, 1993).

Very lipophilic, oil-soluble drugs may also be solubilised in oil-in-water emulsion systems, typically containing 10–20% triglyceride-rich vegetable oils in combination with 1% surfactant (eg phosphatidylcholine (PC)), and 2% glycerol. Inclusion of surface active agents within a formulation can provide several desirable attributes including increased drug solubility and stability through incorporation within micelles, in particular with protein formulations which are prone to aggregation and precipitation. A common formulation example of this type is Cremophor EL® in combination with ethanol. This is used to solubilise paclitaxel, teniposide, valrubicin, and cyclosporine A; all large-molecular-weight drugs which display very low solubility with little or no possibility of ionisation manipulation. Depending on the drug and dose, this formulation is diluted 5 to 100 fold with saline or dextrose prior to administration resulting in the formation of a micellar dispersion. Unfortunately, a broad toxicity profile which includes nephrotoxicity and hepatotoxicity is associated with this system (Thiel *et al.*, 1986 and Tibell *et al.*, 1993).

Liposomes: Potential solutions for poor solubility

The application of liposomes as drug delivery systems is certainly not new, being first proposed by Gregoriadis in the early 70s (Gregoriadis, 1976). Since then, thousands of investigations into the application of these systems for the delivery of a range of moieties, from small molecules to large nucleic acid therapeutics have been undertaken. This is due to their rather unique structure (*Figure 1*), which is composed of lipid bilayer membranes surrounding an inner aqueous core which allows both water soluble and sparingly soluble drugs to be carried within the structure. In addition, unlike some micellar systems tested, iv investigations have demonstrated that liposomes are also

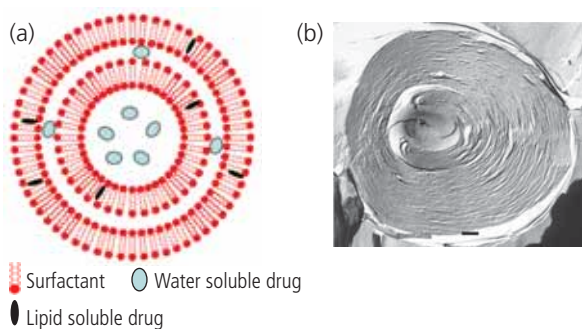


Figure 1 – A schematic representation (a) and an electron micrograph (b) of a multi-layered liposome which can facilitate incorporation of water soluble and lipid soluble drugs within its structure. Bar in (b) represents 100 nm.

able to modify the pharmacokinetic and tissue-distribution patterns of the drug and improve drug delivery to the desired site of action (Lee *et al.*, 1999). There are already several liposome-based drug delivery systems commercially available for drugs including amphotericin B, cytarabine, daunorubicin and doxorubicin. However, only amphotericin B formulations take advantage of the liposomes' solubilising activities, more commonly with the liposome system being exploited for its ability to passively target chemotherapeutic agents.

Considerations When Formulating Liposomes as Solubilising Agents

Despite their current use as solubilising agents, liposome encapsulation efficiency of lipophilic drugs can be very effective with key factors influencing drug loading including log P and lipophilicity (Fresta *et al.*, 1993). However, liposomal incorporation of poorly water-soluble compounds is not only dependent on the physicochemical properties of the drug; factors also include bilayer composition and bilayer volume.

Cholesterol: Not just bad for your heart.

In recent studies from our laboratory (Mohammed *et al.*, 2004), we have shown that both the incorporation and the release of sparingly soluble drugs (e.g. ibuprofen) were influenced by the lipid composition of the liposomes. Phosphatidylcholine (PC; or its derivatives) is the main lipid excipient of several commercially available liposome products due to its non-toxic biodegradable profile. In addition to PC, cholesterol, which is known to influence liposome stability (Gregoriadis, 1993), is often incorporated within liposome bilayers to enhance drug loading and retention within the aqueous compartment. Indeed, the beneficial role of cholesterol within liposomal drug carriers is well recognised with early developmental studies (Gregoriadis and Davis, 1979), demonstrating an optimum of 50% mol/mol cholesterol within a liposome formulation, increasing the stability and reducing the permeability of liposomal bilayers.

The ability of cholesterol to reduce bilayer permeability may be related to cholesterol increasing the packing

densities of phospholipid molecules (Semple *et al.*, 1996) due to the accommodation of cholesterol in the molecular cavities formed by surfactant monomers assembled into vesicles (Devaraj *et al.*, 2002). This is evidenced by surface pressure measurements which show a decrease in effective area per molecule as the cholesterol content of the monolayer is increased (Rogerson *et al.*, 1987). This space-filling action, combined with the ability of cholesterol to complex with phospholipids, can reduce bilayer permeability to small hydrophilic solutes and ions (Papahadjopoulos, *et al.*, 1973). Biophysical studies (Bernsdorff *et al.*, 1997) of phospholipid-cholesterol bilayers have also shown that the addition of 30–50 mol% cholesterol to phosphatidylcholine liposomes can increase the hydrophobicity in the interfacial region of the liposome bilayer, a factor which could influence the incorporation of drugs within the lipid bilayer.

To investigate the effect of cholesterol content on drug solubilisation within liposomes we prepared a range of formulations with increasing cholesterol content (0–50%; Figure 2). In the case of the poorly water-soluble drug ibuprofen, minor improvements in drug loading occurred in PC liposomes containing 20% mol/mol cholesterol content and major reductions in drug loading resulted when the cholesterol content was increased above this. This may be a result of two conflicting factors; the increased hydrophobicity (Bernsdorff *et al.*, 1997), increased bilayer stability and decreased permeability (Gregoriadis and Davis, 1979) with increasing cholesterol content may efficiently trap ibuprofen within the bilayer. Counteracting this, higher amounts of cholesterol may compete with ibuprofen for packing space within the bilayer, limiting its bilayer incorporation.

Liposome Hydrophobic Volume

The choice of PC derivative used within the liposome formulation is also a matter for consideration. Our studies (Table 1) have also demonstrated that solubilisation of sparingly soluble drugs is influenced by the length of the

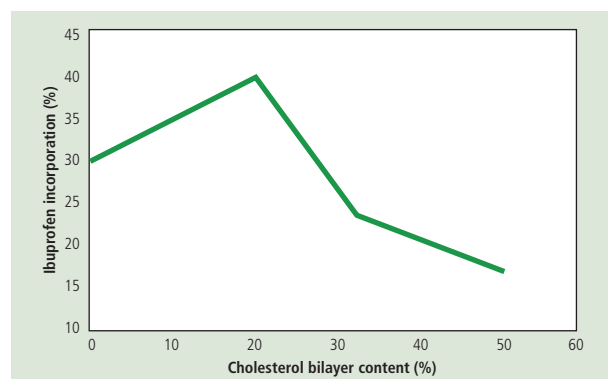


Figure 2 – The effect of cholesterol content on encapsulation of ibuprofen in PC:Chol liposomes. Multi-lamellar vesicles incorporating ibuprofen were prepared from 16 micromol Phosphatidylcholine (PC) and varying cholesterol content (0–50% mol/mol ratio) in the presence of 1.25 mg of ibuprofen. Values denote mean \pm S.D. from at least three experiments.

Lipid	Liposome drug loading (% mol/mol)		
	Sulindac (Log P 3.4)	Ibuprofen (Log P 3.6)	Flurbiprofen (Log P 4.1)
PC	11.5 ± 0.1	10.6 ± 0.2	16.6 ± 0.3
DMPC	12.3 ± 0.3	11.4 ± 0.2	25.3 ± 0.3
DSPC	12.8 ± 0.1	11.7 ± 0.3	29.7 ± 0.2
C ₂₄ PC		15.7 ± 0.2	

Table 1 – The effect of cholesterol content on encapsulation of drugs in PC:Chol liposomes. Multi-lamellar vesicles incorporating either sulindac, ibuprofen or flurbiprofen (1.25 mg) were prepared from 16 µmol Phosphatidylcholine (PC) and varying cholesterol content (0–50% mol/mol ratio). Values denote mean ± S.D. from at least three experiments.

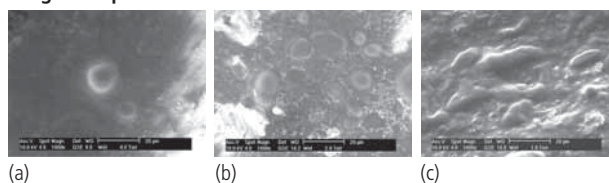
PC alkyl chain used within the liposomal structure. Table 1 demonstrates a trend of increasing bilayer drug loading with increasing lipid chain length. For example, ibuprofen drug loading increases by around 50% when dilignoceroyl phosphatidylcholine (C₂₄PC) is substituted for PC. This increased drug loading capacity of longer alkyl chain lipid bilayers could be attributed to the increased hydrophobic area within the liposome bilayers, similar to the effects previously demonstrated with micellar solubilisation of drugs such as barbiturates (Arnarson and Elworthy, 1980).

However, not only can the liposome formulation have an effect on drug loading, drug loading can also influence the physico-chemical characteristics of the liposomes. Using Environmental Scanning Electron Microscopy (ESEM), we have studied the stability of drug-free and drug-loaded liposomes suspended in phosphate-buffered Saline (pH 7.4) (Mohammed *et al.*, 2004). These studies (Figure 3) revealed that ibuprofen-loaded liposomes were structurally more resistant to destabilisation during dehydration than drug-free liposomes, suggesting a direct effect of drug/lipid binding on liposome bilayer stability. These studies also confirm the utility of ESEM for monitoring the changes in liposome morphology in real time during dehydration, thereby providing an alternative assay of liposome formulation/stability relationships.

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Drug-free liposomes



Drug-loaded liposomes

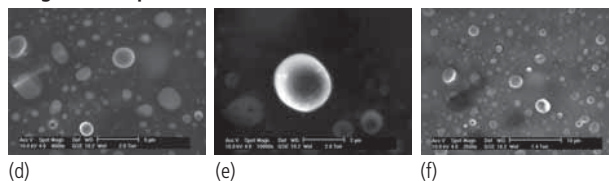


Figure 3 – Environmental scanning-electron micrographs of ibuprofen-loaded MLV (PC:Chol; 80:20% molar ratio) suspended in 0.01 M PBS (pH 7.4). Vesicles were subjected to controlled dehydration in the ESEM sample chamber. At an operating pressure of 4.0 torr (a) drug-free liposomes appear as spherical vesicles. Reduction of the ESEM operating pressure to 2.9 torr (b) results in the liposomes coalescing as the aqueous media evaporates. Further reduction of pressure to 1.9 torr (c) resulted in the drug-free liposomes losing their spherical shape as flattening and spreading occurred to form lipid patches. However, in the case of drug-loaded vesicles spherical liposomal structures remain stable at pressures of 2.0 torr (d, e) and even 1.4 torr (f). (Reproduced with permission, Mohammed *et al.*, 2004)

Conclusion

Liposomes continue to offer novel solutions to many current problems faced by pharmaceutical scientists when formulating and testing new chemical entities. Compared to several of the solubilisation technologies currently employed in formulation, liposomes may offer a wider scope for solubilisation of a range of drugs with the added advantage that the system can also be tailored to provide enhanced drug delivery and targeting.

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