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Preparation and characterization of dehydration–rehydration vesicles loaded with aminoglycoside and macrolide antibiotics

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Abstract

Enhanced activity of liposomes-encapsulated antibiotics against clinical isolates of *Pseudomonas aeruginosa* has been documented with liposomes of low encapsulation efficiency. We sough to construct liposomes with high yield entrapment of aminoglycoside and macrolide antibiotics as well as favorable stability in storage and physiological conditions. Liposome-entrapped aminoglycosides (amikacin, gentamicin, tobramycin) and a macrolide (erythromycin) were prepared by a modified dehydration–rehydration vesicles (DRVs) method, and their particle size and entrapment efficiency were determined. We studied in vitro stability of these vesicles over a 48 h period at 4 and 37 °C in phosphate-buffered saline (PBS) and in plasma at 37 °C. The mean particle size of DRVs loaded with antibiotics varied from 163.37 ± 38.44 to 259.83 ± 11.80 nm with no significant difference in regard with the type of the antibiotics encapsulated. Encapsulation efficiency of DRVs loaded with amikacin, gentamicin, tobramycin, and erythromycin were 29.27 ± 1.17, 33 ± 0.76, 22.33 ± 1.48 and 32.06 ± 0.82% of initial amount of the drug, respectively. These vesicles were stable regardless of the experimental temperature. Indeed, the liposomes retained more than 75% of the initially encapsulated drugs for the study period of 48 h. DRVs incubated in plasma however, released more antibiotics than those incubated in PBS. In conclusion, using this modified DRV method, we obtained small sized vesicles with high yield entrapment for aminoglycoside and macrolide antibiotics. The technique may be utilized to overcome the low encapsulation efficiency associated with aminoglycoside and macrolide antibiotics.
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Keywords: DRVs; Size; Encapsulation efficiency; Stability

1. Introduction

Liposomes have been widely considered as potential drug delivery systems ever since the published observation of Bangham and co-workers (Bangham et al., 1965). Liposomes are colloidal vesicles ranging from few nanometers to several micrometers in diameter with one or more lipid bilayers surrounding aqueous compartments (Vemuri and Rhodes, 1995; Chatterjee and Banerjee, 2002). They are prepared from natural or synthetic phospholipids and cholesterol, however, other lipids or derivatives can also be incorporated as needed (Patel and Sprott, 1999; Chatterjee and Banerjee, 2002). Liposomes are biodegradable, biocompatible, non-toxic and non-immunogenic (Voinea and Simionescu, 2002). They can entrap a wide variety of therapeutic drugs including anti-microbial and anti-cancer drugs (Abraham et al., 2005; Bakker-Woudenberg et al., 2005; Salem et al., 2005). Hydrophilic drugs can be entrapped in aqueous compartments of liposomes, whereas hydrophobic drugs are incorporated in their lipid bilayers (Voinea and Simionescu, 2002). A few liposomal formulations are in clinical practice and some in preclinical trials. For instance, liposomal amphotericin B known as AmBisome is used to treat systemic fungal infections (Veerareddy and Vobalaboina, 2004), while liposome-encapsulated daunorubicin (Doxil) is a first-line cytotoxic drug for advanced virus-associated Kaposi sarcoma in AIDS patients (Theodoulou and Hudis, 2004). Doxil is currently under clinical investigation for treatment of other types of malignancies including breast carcinoma, leukemias and prostate cancer (Flaherty et al., 2004; Theodoulou and Hudis, 2004). Cytotoxicity of these drugs are significantly reduced when administered in liposome forms because of the shift in their biodistribution, pharmacokinetics and therapeutic index

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conferred by the liposomes (Kim and Lim, 2002; Abraham et al., 2005).

Aminoglycosides and macrolides are potent antibiotics with bactericidal activity against Gram-negative and Gram-positive organisms as well as a series of intracellular pathogens. Aminoglycosides are commonly used in combination with β-lactams for treatment of serious bacterial infections (Poole, 2005). More frequently, effective treatment of these infections requires high dosage administration of aminoglycosides which often results in serious toxicity including ototoxicity and nephrotoxicity (Nagai and Takano, 2004). Therefore, administration of aminoglycosides are only limited to patients with severe bacterial infections, especially in immunocompromised patients with mycobacterial infections (Young, 1993; Aiken and Wetzstein, 2002). Macrolides, on the other hand, are perceived as safe drugs and are extensively used worldwide. However, macrolides accumulation in lysosomes could interfere with phospholipase activity and cause phospholipidosis (Horn et al., 1996; Van Bambeke et al., 1998; Montenez et al., 1999). In addition, high doses of macrolides cause hepatic injury in humans and in experimental animals as well (Zafrani et al., 1979; Venkateswaran et al., 1997).

Encapsulation of aminoglycosides and macrolides into liposomes has been attempted by our group as well as several other investigators as a mean of altering drug biodistribution, reducing their toxicity and increasing their therapeutic index (Omri et al., 1994; Bakker-Woudenberg et al., 1995; Beaulac et al., 1996; Lutwyche et al., 1998; Cordeiro et al., 2000; Schiffelers et al., 2001a,b; Marier et al., 2002, 2003; Mugabe et al., 2005). One of the limitations of liposomes-based aminoglycoside and macrolide antibiotics is their low encapsulation efficiency, which results in formulations with low drug-to lipid ratio. This, in turn, will require high amount of liposomal formulations to achieve desirable therapeutic dose, a feature that defeat the purpose.

We have previously developed various liposomal formulations containing gentamicin with enhanced antibacterial activity against resistant strains of *Pseudomonas aeruginosa* isolated from the lungs of cystic fibrosis patients. These formulations altered the susceptibility of *P. aeruginosa* from highly resistant to either intermediate or susceptible to this drug (Mugabe et al., 2005). However, encapsulation efficiencies of these formulations were limited to only 5%. The present study was undertaken to evaluate the encapsulation efficiency and in vitro stability of aminoglycoside and macrolide antibiotics entrapped in dehydration/rehydration vesicles (DRVs) by a modified protocol.

2. Materials and methods

2.1. Chemicals

Amikacin, gentamicin, tobramycin and erythromycin were obtained from Fisher Scientific (Ottawa, Ont., Canada). Cholesterol and Triton X-100 were purchased from Sigma–Aldrich Canada Ltd. (Oakville, Ont., Canada). 1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) was obtained from Northern Lipids (Vancouver, BC, Canada). All other chemicals were purchased from Sigma–Aldrich.

2.2. Organisms

We used two laboratory strains of *Bacillus subtilis* (ATCC 6633) and *Staphylococcus aureus* (ATCC 29213) as reference organisms in microbiological assay. The organisms were stored at −80 °C in Mueller–Hinton broth (Becton Dickinson, Cockeysville, MD, USA) supplemented with 10% (v/v) glycerol. For experimentation, an overnight culture in Mueller–Hinton broth was used to prepare a bacterial solution equivalent to the 0.5 McFarland tube about 1.5×10^8 bacteria/ml.

2.3. Normal human pooled plasma

A pool of normal citrated human plasma from a minimum of 20 healthy individuals was obtained from Precision-Biologic (Dartmouth, NS, Canada). Aliquots of 10 ml/vial were stored at −80 °C.

2.4. Preparation of dehydration–rehydration vesicles (DRVs)

Liposomes composed of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and cholesterol in a molar ratio of 2:1 (lipid to cholesterol). We modified the dehydration–rehydration method of Kirby and Gregoriadis (Kirby and Gregoriadis, 1984), as follows. A 50 μmol of DPPC and 25 μmol of cholesterol were dissolved in 1 ml of chloroform in a 50 ml round-bottomed flask and dried to a lipid film with a rotary evaporator (R 205, Brinkman) at 50 °C under controlled vacuum (V-800, Brinkman). The lipid film was flashed with nitrogen gas to eliminate traces of chloroform prior to rehydration with 2 ml of distilled water/sucrose (1:1, w/w, sucrose to lipid). Sucrose was used to stabilize the liposomes during freeze drying. The lipid suspensions were vortexed for 2 min to form multilamellar vesicles (MLVs) and then sonicated for five minutes (cycles of 40 s run and 20 s pause) in an ultrasonic dismembrator bath (Model 500, Fisher Scientific). The resulting mixtures were centrifuged at low speed ($400 \times g$, 10 min at 4 °C) to remove large vesicles. The suspension of small unilamellar vesicles (SUVs) was then mixed with 1 ml (40 mg/ml) of the target antibiotic. The mixture was then frozen (−20 °C) and immediately freeze dried overnight (Freeze Dry System model 77540, Labconco Corporation, Kansas City, MO, USA). The powdered formulations were stored at 4 °C until use. For rehydration, we added 200 μl of distilled water, vortexed, and incubated for 30 min at 50 °C. We repeated the step with 200 μl phosphate-buffered saline (PBS, pH 7.4) and after incubation period, 1.6 ml of PBS was added and the mixture was vortexed and incubated for another 30 min at 50 °C. Excess unencapsulated drug was removed following three rounds of PBS wash ($18{,}300 \times g$ for 15 min at 4 °C). The pellet was then resuspended in PBS and encapsulation efficiency was quantitated by agar diffusion microbiological assay after lipid vesicles were lysed with 0.2% Triton X-100. We must point out that this level of Triton X-100 (0.2%) has no effect on the performance of the assay and that this is the only experiment where we used a detergent.

2.5. Particle size of DRVs

The mean diameter of DRVs and the polydispersity index were determined by photon correlation spectroscopy (PCS) with the use of Submicron Particle Sizer, Model 270 (Nicomp, Santa Barbara, CA, USA). All liposomes preparations were analyzed before and after freeze-drying/rehydration (F.D.) and Gaussian distribution was chosen based on our facility standard. Polydispersity index of 0.0 represents a homogeneous particle population while 1.0 indicates a heterogeneous size distribution in the DRV preparations.

2.6. Encapsulation efficiency of antibiotics loaded into DRVs

Encapsulation efficiency was determined as the percentage of antibiotic incorporated into DRVs relative to initial total amount of drug in solution. Encapsulation efficiency was calculated using the equation below:

$$\text{encapsulation efficiency}\,(\%) = \frac{C_{\text{DRVs}}}{C_{\text{sol}}} \quad (1)$$

where C_{DRVs} is concentration of the antibiotic entrapped in DRVs and C_{sol} is the initial concentration of the antibiotic added into the mixture.

2.7. Quantification of antibiotics

The concentrations of antibiotics incorporated into DRVs were measured by agar diffusion assay using laboratory strains of *S. aureus* (ATCC 29213) as indicator organism for gentamicin and tobramycin and *B. subtilis* (ATCC 6633) as indicator organism for amikacin and erythromycin. Briefly, we used an overnight culture of the organisms in Mueller–Hinton broth to prepare a bacterial solution equivalent to a McFarland 0.5 (1.5×10^8 bacterial/ml). The bacterial suspension in warm (50 °C) Muller–Hinton agar was then poured into a sterile steel plate (440 mm × 340 mm) and left to solidify for 1 h at room temperature. Wells of 5 mm diameter, made with a well puncher, were filled with 25 μl of samples or standard solutions. The plate was covered and incubated for 18 h at 37 °C. We then measured the inhibition zones and, the average of duplicate measures was used in data analysis. A standard curve was constructed with known concentrations of free gentamicin (0.0039–8 mg/ml) and was utilized to calculate concentrations of the entrapped antibiotics that were released from the liposomes by 0.2% Triton × 100. The minimum detection limit of the assay for amikacin, gentamicin, tobramycin and erythromycin was 15.6, 7.8, 3.9 and 31.2 ng/ml, respectively.

2.8. Stability of DRVs loaded with antibiotics

The stability of DRVs loaded with antibiotics was evaluated in PBS and in normal human pooled plasma as we previously described (Mugabe et al., 2005). Briefly, appropriate amount of liposomes was suspended in PBS (pH 7.4) and incubated at 4 or 37 °C with a mild agitation (100 rpm). Likewise, to mimic the physiological conditions, normal human pooled plasma was supplemented with DRVs and incubated at 37 °C with a mild agitation. After incubation periods of 2, 4, 6, 8, 24 and 48 h, samples were removed and centrifuged (18,300 × *g*, 15 min at 4 °C) to remove the leaked drugs. Drugs concentrations in the supernatants were determined by agar diffusion assay as described above. We evaluated the DRVs stability by determining the amount of drug released over a 48 h study period. Drug release was expressed as percentage of the concentration of the entrapped antibiotic measured by agar diffusion assay.

2.9. Data analysis

The data are expressed as mean ± S.E.M. of three independent experiments. Comparisons were made by paired Student's *t*-test and $P \leq 0.05$ was considered significant. For multiple comparisons within and between groups, ANOVA with the two-tailed Dunnett's post-test analysis was used.

3. Results

3.1. Particle size and polydispersity index of DRVs

The mean particle size of DRVs loaded with target antibiotics varied from 163.37 ± 38.44 to 259.83 ± 11.80 nm. As shown in Table 1, although chemical nature of the antibiotics had an impact on the size of DRVs, these differences were not statistically significant ($P > 0.05$). Also, the process of freeze-drying did not affect the particle size of rehydrated liposomes as there was no significant difference in particle size before and after freeze-drying ($P > 0.05$). The polydispersity index ranged from 0.60 ± 0.01 to 0.74 ± 0.01, indicating the presence of a heterogeneous DRVs population (Table 1).

3.2. Encapsulation efficiency of antibiotics loaded into DRVs

The encapsulation efficiency of aminoglycoside and macrolide antibiotics that represents the amount of antibiotic incorporated into DRVs relative to the initial concentration of drugs used in preparation of liposomes is shown in Table 1. In general, the encapsulation efficiency for each of the target antibiotics was more than 20% of the initial amount of the drug. Differences in the encapsulation rates of amikacin (29.27 ± 1.17%), gentamicin (33 ± 0.76%) and erythromycin (32.06 ± 0.82%) were not remarkable ($P > 0.05$). Whereas, the encapsulation rate of tobramycin (22.33 ± 1.48%) was significantly lower than that of amikacin, erythromycin ($P < 0.01$) and gentamicin ($P < 0.001$).

3.3. Stability of DRVs loaded with antibiotics

The stability of DRVs loaded with aminoglycoside and macrolide antibiotics was evaluated in PBS at 4 °C (storage temperature), 37 °C (body temperature), as well as in normal human pooled plasma (physiological condition) for a period of study of 48-h. Collectively, the DRVs stored at 4 °C were more

Table 1
Particle size, polydispersity index (P.I.) and encapsulation efficiency of aminoglycoside and macrolide antibiotics into dehydration–rehydration vesicles (DRVs)

Liposome composition	Average diameter (nm)		P.I.	Encapsulation efficiency (%)
	Before F.D.	After F.D.		
DPPC-CHOL-AMK	159.70 ± 39.82	163.37 ± 38.44	0.60 ± 0.01	29.27 ± 1.17
DPPC-CHOL-GEN	171.73 ± 13.32	205.40 ± 28.13	0.74 ± 0.01	33.00 ± 0.76
DPPC-CHOL-TOB	240.63 ± 16.85	259.83 ± 11.80	0.69 ± 0.02	22.33 ± 1.48
DPPC-CHOL-ERT	155.85 ± 25.08	194.83 ± 42.74	0.67 ± 0.02	32.06 ± 0.82

Particle size and polydispersity index (P.I.) of liposomes were determined by PCS with the use of Submicron Particle Sizer. All liposomes preparations were analyzed before and after freeze-drying/rehydration (F.D.) and Gaussian distributions were recorded. P.I, gives the measurement of homogeneity of dispersion, ranging from 0.0 (homogenous) to 1.0 (heterogeneous). The concentrations of antibiotics incorporated into DRVs were measured by agar diffusion assay using laboratory strains of *S. aureus* (ATCC 29213) as indicator organism for gentamicin (GEN) and tobramycin (TOB) and *B. subtilis* (ATCC 6633) for amikacin (AMK) and erythromycin (ERT). Encapsulation efficiency was determined as the percentage of antibiotic incorporated into DRVs relative to initial total amount of drug in solution. Results are given as mean ± S.E.M. of three separated experiments.

stable than those incubated at 37 °C, regardless of their antibiotic contents or the solvents. For instance, DRVs containing amikacin retained 99.15 ± 0.85% of the drug at 4 °C compared to 79.78 ± 2.50% ($P < 0.001$) at 37 °C at the end of a 48-h study period. On the other hand, DRVs carrying various antibiotics behaved differently when they stored in different medium, i.e., PBS or serum, at 37 °C. For example, as shown in Fig. 1, the DRVs incubated in PBS retained significantly ($P < 0.001$) more amikacin than those incubated in plasma (79.78 ± 2.50% versus 51.97 ± 2.08%). In case of gentamicin and tobramycin, however, DRVs were equally stable in both environments. Furthermore, as shown in Figs. 2 and 3, the DRVs retained more of these antibiotics than amikacin (80% versus 52%).We evaluated the stability of DRVs containing a macrolide (erythromycin) in similar conditions as described above. As shown in Fig. 4, the DRVs containing erythromycin retained 100% of the entrapped erythromycin at 4 °C (the release kinetics of these vesicles were under the detection limit which was 31.2 μg/ml). At 37 °C, however, the DRVs in serum environment lost about 50% of their content in 2 h, after which the drug release was stabilized through

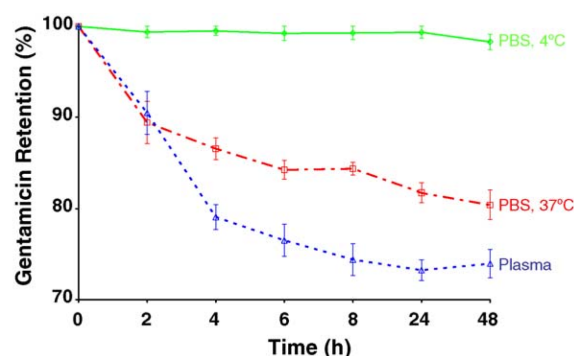

Fig. 2. In vitro stability of dehydration–rehydration vesicles (DRVs) entrapped gentamicin. Liposomes-entrapped gentamicin were incubated in PBS at 4 °C (◊), 37 °C (□) and plasma (△) with a mild agitation. At times indicated, samples were centrifuged at 18,300 × *g* for 15 min and gentamicin concentrations in the supernatants were determined by the agar diffusion microbiological assay. Results are given as mean ± S.E.M. of three separated experiments. DRVs entrapped gentamicin retained significantly more antibiotics than those incubated in PBS at 37 °C ($P < 0.05$) or in plasma ($P < 0.01$) at the end of the period of study. However, DRVs incubated at 37 °C or plasma retained comparable amount of gentamicin through out the period of study.

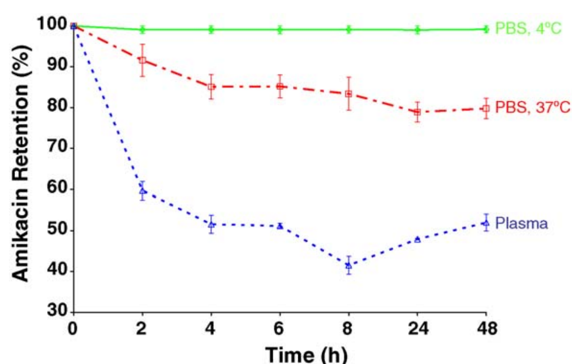

Fig. 1. In vitro stability of dehydration–rehydration vesicles (DRVs) entrapped amikacin. Liposomes-entrapped amikacin were incubated in PBS at 4 °C (◊), 37 °C (□) and plasma (△) with a mild agitation. At times indicated, samples were centrifuged at 18,300 × *g* for 15 min and amikacin concentrations in the supernatants were determined by the agar diffusion microbiological assay. Results are given as mean ± S.E.M. of three separated experiments. DRVs stored at 4 °C retained significantly more antibiotics (99.15 ± 0.85%) than those incubated either 37 °C (79.78 ± 2.50%, $P < 0.001$). On the other hand, DRVs incubated at 37 °C retained significantly ($P < 0.001$) more amikacin than those incubated in plasma (51.97 ± 2.08%) at the end of a 48-h period of study.

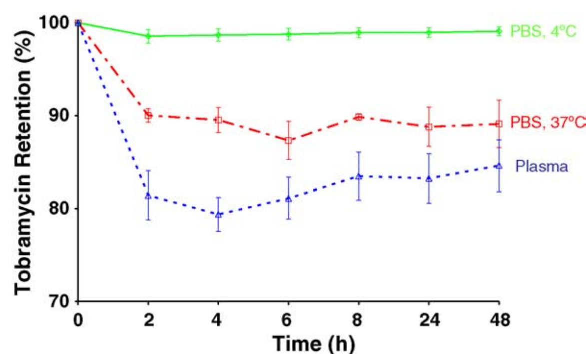

Fig. 3. In vitro stability of dehydration–rehydration vesicles (DRVs) entrapped tobramycin. Liposomes-entrapped tobramycin were incubated in PBS at 4 °C (◊), 37 °C (□) and plasma (△) with a mild agitation. At times indicated, samples were centrifuged (18,300 × *g* for 15 min) and tobramycin concentrations in the supematants were determined by the agar diffusion microbiological assay. Results are given as mean ± S.E.M. of three separated experiments. DRVs stored at 4 °C retained 99.08 ± 0.48% of initial encapsulated tobramycin at the end of a 48-h period of study. DRVs incubated in PBS at 37 °C retained significantly more antibiotics ($P < 0.01$) than those in plasma after 4-h incubation, after this period the release kinetics of both these DRVs were similar ($P > 0.05$) until the end of the period of study.

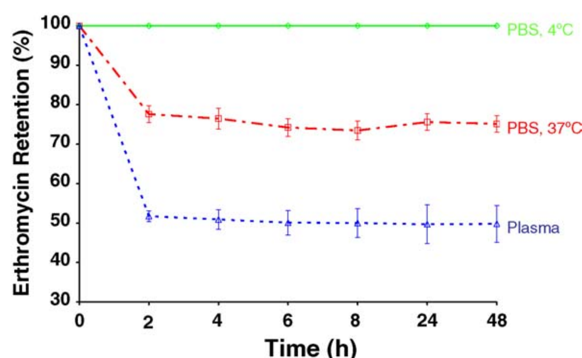

Fig. 4. In vitro stability of dehydration–rehydration vesicles (DRVs) entrapped erythromycin. Liposomes-entrapped erythromycin were incubated in PBS at 4 °C (◊), 37 °C (□) and plasma (△) with a mild agitation. At times indicated, samples were centrifuged (18,300 × *g* for 15 min) and erythromycin concentrations in the supernatants were determined by the agar diffusion microbiological assay. Results are given as mean ± S.E.M. of three separated experiments. DRVs store at 4 °C retained 100% of the initial entrapped erythromycin. DRVs incubated at in PBS at 37 °C retained significantly more antibiotics ($P < 0.05$) than those in plasma for the entire period of study.

out the study period of 48 h. The drug release in PBS was also stable after a modest drop of about 20% in the first 2 h.

4. Discussion and conclusion

We sought to construct liposomes with high yield entrapment of aminoglycoside and macrolide antibiotics as well as a favorable stability. In this study, we have demonstrated that aminoglycoside and macrolide antibiotics could be efficiently encapsulated in liposomes composed of DPPC–cholesterol (molar ratio 2:1) prepared by a modified method that produces dehydration–rehydration vesicles termed DRVs. The choices of lipid composition as well as the lipid–cholesterol ratio used to prepare DRVs were based on our previous study (Mugabe et al., 2005).

The DRVs loaded with aminoglycoside and macrolide antibiotics produced in this study, however, were smaller (163.37 ± 38.44 to 259.83 ± 11.80 nm) than those we reported in the previous study (408 ± 28 to 418 ± 21 nm) ($P < 0.05$). The difference in size of these formulations is due to the protocols, i.e., sonication versus freeze/dry methods, which we utilized to prepare these liposomes. The sonication technique produces a mixture of small unilamellar vesicles (SUVs) and multilamellar vesicles (MLVs). The freeze/dry method utilized in this study, however, eliminates the large vesicles and results in a uniform suspension of SUVs. In addition, we used sucrose to prevent the conversion of the SUVs to MLVs during dehydration process (Zadi and Gregoriadis, 2000; Kawano et al., 2003). As demonstrated in Table 1, the process of freeze-drying had no significant effect on the particle size of rehydrated liposomes, indicating the absence of vesicle fusion. This may be due to the presence of sucrose in our formulation as it has been well documented that sugar has the ability to prevent vesicle fusion during freeze-drying of liposomes. For instance, Kawano et al. (2003) prepared pirarubicin (an anticancer agent derived from doxorubicin) loaded into DRVs in the presence or absence of sugar and found that the liposomes without sugar were significantly larger than those prepared with sugar (1000 nm versus 400 nm). Others have reported similar observations on various liposomal drug formulations (Zadi and Gregoriadis, 2000; Komatsu et al., 2001; Aso and Yoshioka, 2005; Glavas-Dodov et al., 2005). The ability of sugars to prevent vesicle fusion has been attributed to their formation of a stable glassy state as well as a direct interaction between sugars and the polar head of phospholipids (Crowe and Crowe, 1988; Crowe et al., 2003; Wolkers et al., 2004a,b). As such, disaccharides such as sucrose are better liposome stabilizer than either mono-or polysaccharides (Crowe and Crowe, 1988; Hincha et al., 2002; Kawano et al., 2003; Ricker et al., 2003; Wolkers et al., 2004a). Our modified dehydration–rehydration method also improves encapsulation efficiency of aminoglycoside and macrolide antibiotics. Our group as well as other investigators reported much lower encapsulation efficiency (4–5% versus 22–33% in this study) for similar formulations that were prepared by sonication methods (Cajal et al., 1992; Mugabe et al., 2005). Likewise, Frezard et al. have reported higher encapsulation efficiency of meglumine antimoniate (antiprotozoals agent to treat leishmaniasis) in DRVs (42 ± 2%) than in MLVs (12.5 ± 1%) and freeze-dried empty Hposomes (FDELs; 31 ± 3%) methods (Frezard et al., 2000). The ability of high-yield entrapment of drugs into DRVs is attributed to the fact that during the dehydration process, the lipids (or SUVs) interact intimately with the drugs due to the reduced hydrophobic forces. This allows the entrapment of a relatively large proportion of the drugs in the aqueous compartments of the DRVs following the controlled rehydration process (Zadi and Gregoriadis, 2000). Whereas, there is no such intimate interactions between lipids and the drugs in the sonication method. Instead, anhydrous lipid becomes hydrated and round off into vesicles, hence encapsulating a small portion of aqueous phase. Other investigators have attempted to optimize the encapsulation of drugs in liposomes using different techniques with no success. For instance, Ruijgrok et al. have attempted to increase the encapsulation efficiency of gentamicin into liposomes (composed of PEG-DSPE/PHEPC and cholesterol) by using various techniques such as passive and active loading and the highest encapsulation efficiency of gentamicin reported was only 4.0 ± 0.4%, obtained by passive loading technique (Ruijgrok et al., 1999). Although, the encapsulation efficiencies reported here are significantly higher than those reported previously (Omri et al., 1995; Lutwyche et al., 1998; Ruijgrok et al., 1999; Mugabe et al., 2005), even higher encapsulation efficiencies of aminoglycosides have been reported by other investigators (Cajal et al., 1992; Beaulac et al., 1999; Zhang and Zhu, 1999). For instance, Zhang et al. have reported encapsulation efficiency of amikacin up to 95.3 ± 0.6% in liposomes composed by soybean lecithin and cholesterol (Zhang and Zhu, 1999). Their method of liposome preparation, however, differ from ours in that, they used a combination of proliposomes technique with freeze-drying. The potential draw back with this method is the large vesicles ranging in microns, which are pharmacologically unfavorable due to their fast elimination by the reticuloendothelial system (Turanek et al., 1997).

Higher encapsulation efficiency (57.41 ± 2.06%) for gentamicin has also been reported in liposomes composed of egg phosphatides and cholesterol (2:1, w/w) prepared by the DRV method (Cajal et al., 1992). However, this formulation lacks a stabilizing sugar such as sucrose which prevents liposomes fusion during freeze-drying. Sucrose, on the other hand, is known to reduce encapsulation rate of drugs in DRVs, if present at high ratio (i.e. sucrose/lipid 5:1, w/w) (Zadi and Gregoriadis, 2000). Therefore, we chose to use a low ratio of sucrose to lipid in our formulation (1:1, w/w). In preliminary study, this concentration of sucrose prevented vesicle fusion during dehydration/rehydration process without affecting the encapsulation rates of the drugs in DRVs.

The stability of liposomes is another important factor in the development of an effective drug delivery system. Therefore, we evaluated the stability of our liposomal formulations under different environment to mimic physiological conditions. Our data indicate that these vesicles are stable regardless of the experimental temperature (4 or 37 °C). Indeed, the liposomes retained more than 75% of the initially encapsulated drugs for the study period of 48-h at 37 °C. The outcomes were even better at the lower temperature (>95% retention rate for 2 days). This phenomenon may be due to the change in the phase transition from gel to liquid-crystalline (Tm_{DPPC} = 41 °C) rather than alteration in particle size of liposomes. Furthermore, we did not detect any significant alteration in the liposomes size during or at the end of the stability study, indicating that the vesicles fusion or aggregation alone could not explain the drug release data (data not shown). Interactions between DRVs and plasma components such as albumin and high density lipoproteins destabilize liposomes and results in more drug leakage (Guo et al., 1980; Liu et al., 1990; Vitas et al., 1996; Choice et al., 1999), a phenomenon that occurred in this study as well. Our formulation, however, performed better when compared to other similar products (Beaulac et al., 1998), in part, due to the inclusion of cholesterol in our formula (Vitas et al., 1996). In order to avoid stability problems during storage, such as those encountered with aqueous liposome suspensions, DRVs can be stored indefinitely as dried power (lyophilized) that can be rehydrated with water or PBS just before use (Kirby and Gregoriadis, 1984; Frezard et al., 2000). More importantly, the simplicity of this technique makes the large-scale manufacturing of the liposomal formulations feasible.

In conclusion, we have developed a modified DRV method by which we obtained small sized vesicles with high yield entrapment for aminoglycoside and macrolide antibiotics. These vesicles were found to be stable regardless of storage temperature, although they performed better at 4 °C.

This technique can be used to overcome the low encapsulation efficiency associated with aminoglycoside and macrolide antibiotics in liposomes. Our research is now focused on improving the stability of these products at physiological condition.

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