Topical anesthetics remain a powerful tool for pain relief prior to cutaneous procedures. They are frequently used by dermatologists to decrease the pain associated with surgical procedures, laser pulses, or soft tissue augmentation, and with the emergence of new surgical and laser techniques, the need for more effective topical anesthesia continues to grow.

Each local anesthetic has a hydrophobic and a hydrophilic portion. The hydrophilic portion is a weak amine base and exists in both ionized and un-ionized forms. The ionized protonated form predominates at lower pH levels, and the unionized form predominates at higher pH levels. Only the non-ionized form can penetrate neuronal membranes to reach receptor sites on the internal surface of sodium channels (1).

Topical anesthetics have been used for many years on mucosal surfaces because of the relative ease of penetration of topical agents through mucosa. However, the keratinized stratum corneum has been a major barrier to the use of topical anesthetics on normal skin (2). Early formulations of local anesthetics resulted in significant dermatitis, systemic toxicity, or inadequate local analgesia.

Various topical anesthetic agents such as benzocaine, tetracaine, lidocaine, dyclonine, proxamine and dibucaine have been used in various formulations for skin and mucosal applications. These proved clinically useful for the temporary relief of pain, for mucosal anesthetic effect before endoscopic procedures and itching associated with burns, insect bites, postoperative wounds, and minor skin irritations. Among these topical anesthetics and of particular interest is dibucaine, a potent long acting local anesthetic.

Few studies tackled the incorporation of dibucaine in novel topical drug delivery systems to target the drug to the dermal region and eliminate its toxic systemic action. Lai and Roberts (3) investigated the epidermal application of dibucaine through iontophoresis. Other investigators studied the incorporation of dibucaine in biodegradable polymer matrices (4), biodegradable microparticles (5) and multilamellar liposomal vesicles (6). Although dibucaine multilamellar vesicles have been formulated by Mezei et al., no characterization or stability parameters have been investigated in such study, as the main local anesthetic deployed in that study was tetracaine. Also, no topical liposomal dibucaine product has yet been marketed.

Liposomes have shown great potential as topical drug delivery system and proved to be clinically superior to conventional dosage forms (7).

**Abstract:** Formulation of local anesthetics in liposomal topical drug delivery system could provide a sustained and localized anesthesia. The aim of this study was to develop a liposomal dibucaine base (DB) local anesthetic delivery system. DB-loaded multilamellar vesicles (MLVs) were prepared through varying lipid composition, induced charge and pH of the hydration medium. Liposomes were characterized for morphology, size, entrapment efficiency (EE), in vitro drug release and stability including leakage stability. The percentage of drug entrapped in liposomes was found to be hydration medium pH dependent and charge dependent and more pronounced for negatively charged liposomes prepared using hydration medium of pH 9. In vitro release studies of liposomes have shown a sustained release of entrapped dibucaine compared to control solution. Results revealed that adjusting the various formulation variables of dibucaine base MLVs could yield stable and effective topical liposomal local anesthetic formulations.

**Keywords:** Dibucaine base, stearylamine, dicetylphosphate, cholesterol, in vitro release, release stability, multilamellar vesicles, encapsulation efficiency, sink conditions, hydration medium.

---

* Correspondence e-mail: nounousomes@gmail.com
Liposomes of different size and characteristics usually require different methods of preparation. Multilamellar vesicles (MLVs) are by far the most widely studied type of liposomes and are favored for the topical delivery of hydrophobic drugs (8). The most widely used method for preparation of multilamellar liposomes is the thin film hydration technique, first introduced by Bangham et al. (9), in which a thin film of lipids is hydrated with an aqueous buffer.

Although incorporation of dibucaine base in liposomal formulations is a promising tool for sustained dermal local anesthesia, preparation and in vitro characterization of dibucaine base is challenging due to the hydrophobic nature of the drug which hinders proper in vitro drug release evaluation.

The objective of this paper was the preparation, characterization and stability testing of multilamellar liposomes containing dibucaine base. Different formulation variables of dibucaine liposomal dispersions were investigated to achieve maximum drug entrapment and highest stability. These include the effect of the lipid: cholesterol molar ratio, charge induction and pH of the aqueous buffer system used in the preparation of dibucaine liposomal dispersions. The prepared liposomes were characterized for their morphology, vesicle size, entrapment efficiency, in vitro drug release and physical stability.

EXPERIMENTAL

Chemicals

Dibucaine base was obtained from Ward Blenkinsop Co. (London, England), Cholesterol 99% extra pure was purchased from S.d. Fine-Chem Ltd. (Mumbai, India). L-α-phosphatidylcholine, type X-E: from dried egg yolk, stearylamine and dicetylphosphate were from Sigma Chemical Co. (St. Louis, USA).

Spectra/Por® dialysis membrane 12,000–14,000 molecular weight cut off was received from Spectrum Laboratories Inc. (USA).

All other materials and solvents were of analytical grade and double-distilled water was used.

Apparatus

For analysis, a Shimadzu UV visible spectrophotometer, model UV-1601 PC (Japan) was used. Other instruments include Rotavapor, Type R 110 (Buchi, Switzerland), AND sensitive electric balance model 310 (A&D Company Ltd., Japan), (Sigma Laborzentrifugen GmbH, Germany) refrigerated centrifuge 3K-30, JEOL scanning electron microscopy, model JEM-100S (Jeol, Japan), Cilas Laser diffraction particle size analyzer (Model 1064 Liquid) and Electrolab tablet dissolution tester USP 24, model TDT-06N (Electrolab Gansons Engineering, PVT Ltd., Mumbai, India).

Liposome preparation

The lipid components consisting of phosphatidylcholine either alone or in different molar ratios with cholesterol and dicetylphosphate or stearylamine, were weighed into longnecked pear-shaped quick-fit round-bottom flask and dissolved in 5 mL chloroform/methanol (7:3, v/v). Dibucaine base dissolved in 3 mL of the same solvent mixture was added to the lipid solution. The organic solvent was removed under reduced pressure at 43°C using a rotary evaporator. The resulting thin lipid film was slowly hydrated using 8 mL of either phosphate buffer pH 5.6 or bicarbonate buffer pH 9.0.

The process of hydration involved rotation at a low speed at 43°C in the rotary evaporator (with no vacuum) for 30 min followed by hand shaking for 15 min at 43°C in a thermostatically controlled water bath. The resulting pale liposomal milky dispersion was left to mature overnight at 4°C.

A total of 12 dibucaine liposomal dispersions (Table 1) were prepared differing from each other in lipid composition and hydration medium (pH and buffer species). Each dispersion was prepared in triplicate.

Separation of free dibucaine

This was achieved by centrifugation of the prepared dispersions at 16500 rpm (27800 X g) for 90 minutes at –5°C (Beckman model J2-21 centrifuge). The resulting liposomal concentrate was washed twice each with 5 mL of either phosphate buffer pH 5.6 or bicarbonate buffer pH 9.0 and recentrifuged for further 90 min. The resulting liposomal concentrates were refrigerated.

Entrapped drug was determined by lysis of liposomes with chloroform/methanol (7:3, v/v). Dibucaine concentration was determined spectrophotometrically at 241 nm (10) using the lysis mixture as blank, as described by Mezei et al. (6).

CHARACTERIZATION OF THE PREPARED DIBUCaine LIPOSOMES

Electron microscope examination (11)

Dibucaine liposomal dispersion (D₉pH9) was examined by transmission electron microscopy.

The sample was examined by transmission electron microscopy at 100,000 X magnification po-
wer through negative staining using 2% w/v ammonium molybdate aqueous solution at 80 kV.

Vesicle size analysis (11)

Liposome vesicle size was determined using a laser diffraction particle size analyzer operated at a wavelength 780 nm, and at a measuring range 1–150 µm. The sample (liposomal dispersion in the corresponding buffer), diluted with the same buffer system was stirred during the measurement. Sizing was carried out on freshly prepared liposomal dispersions. Results were recorded as median vesicle size and cumulative percent frequency under size.

In vitro release of dibucaine from liposomal dispersions

Drug release from liposomes was studied using a dialysis method. Dialysis bags were spectra/Por® 2 of 12,000–14,000 Da molecular weight cut off. The bags were soaked before use in distilled water at room temperature for 12 h. The bags were suspended in the release medium and tested for leakage. The final length of the bag after tying was 8 ± 0.2 cm. The dialysis bag was attached horizontally fully stretched to the paddle by attaching a stainless steel part to allow fixing the dialysis bags to it (Figures 1 and 2).

Dibucaine liposomal concentrate (equivalent to 2 mg of dibucaine base) dispersed in mL of either phosphate buffer pH 5.6 or bicarbonate buffer pH 9 was filled in a dialysis bag of 10 cm initial length and 6.4 mm diameter. The bag was closed at both ends with cotton thread and tested for leakage. The final length of the bag after tying was 8 ± 0.2 cm. The dialysis bag was attached horizontally fully stretched to the paddle and immersed in the release medium (phosphate buffer pH 5.6 containing 7% v/v propylene glycol and 25% v/v methanol). The bag was fully immersed under the surface. The temperature was set at 32 ± 0.2°C and the speed of rotation of the paddles at 100 rpm.

Control bags were prepared and tested along with the liposomal dispersions. The control bags each contained 2 mg of dibucaine base dissolved in mL of the release medium. Aliquots of the release medium were withdrawn for analysis at different time intervals and replaced with fresh medium. Release runs were continued for 12 h. The absorbance of the collected samples diluted as necessary with release medium was measured at λmax 244 nm. The results recorded are the mean value of three runs carried out for each liposome concentrate.

A representative liposomal dispersion was examined by electron microscopy before and after the release run.

Stability testing of dibucaine liposomal dispersions

Visual stability rating

Six of the dibucaine liposomal dispersions (5 mL each) prepared by diluting the concentrates with the corresponding buffer were stored at room temperature for 12 month study period at the end of which they were examined visually for evidence of phase separation, and microscopically for signs of coalescence. The rating method used was that suggested for disperse systems by Hanna (12).

Physical stability of dibucaine liposomal dispersions

Possible leakage of dibucaine from multilamellar vesicles was monitored during storage of two of the liposomal concentrates. Changes in release profile of dibucaine were taken as indicator of instability. Release profiles were obtained as previously detailed for a freshly prepared liposomal dispersion (zero time) and storage for 1, 2 and 4 weeks in well closed tubes at 4°C.

RESULTS AND DISCUSSION

Mean values of dibucaine entrapment efficiency in multilamellar vesicles (MLVs) obtained ranged from 20.57% ± 2.23% to 91.76% ± 3.47% (Figure 3). The trends observed in these results were lower entrapment efficiency for the phosphate buffer pH 5.6 (as the hydration medium) compared to the bicarbonate pH 9.0 medium, higher entrapment efficiency for the negatively charged compared to neutral and positively charged liposomes.

Phosphate buffer pH 5.6 and bicarbonate buffer pH 9.0 were used as hydration medium in the preparation of different batches of liposomes. Liposomes prepared using bicarbonate buffer pH 9.0 showed higher entrapment efficiency compared to liposomes prepared using phosphate buffer pH 5.6 irrespective of liposome charge and cholesterol content (Figure 3). For example, an increase in entrapment efficiency of neutral liposomes from 26.97% ± 1.5% to 79.59% ± 3.12% resulted from using bicarbonate buffer instead of phosphate buffer under the same conditions (DpH5 compared to DpH9; Figure 3).

Comparable results were obtained with negatively and positively charged liposomes; again higher entrapment efficiency resulted with the bicarbonate buffer. This is consistent with the results of Mezei et al. (6) and Surewicz and Leyko (13).

The partition coefficient of the drug between the lipid layer and the hydration medium is a key issue for the successful preparation of dibucaine base multilamellar liposomes, which influences the dibucaine entrapment efficiency in the liposomal vesi-
cles. When the liposomes are prepared by mixing the hydrophobic drug with lipids, the drug will eventually partition, during the hydration step, to an extent depending on the partition coefficient of the drug and the phase volume ratio of water to bilayer. The higher the drug partitioning into the aqueous hydration medium, the lower will be the entrapment efficiency. The effect of drug partition coefficient was investigated by varying the pH of the hydration medium. The bicarbonate buffer pH 9 favored unionized drug species, high lipid/water partition coefficient and high entrapment in liposomes compared to pH 5.6 which favored dibucaine base protonation and sequestration in the hydration medium, leading to lower entrapment efficiency (reported dibucaine pKa is 8.15).

Based on the knowledge of drug saturation solubility in the hydration medium, it was possible to theoretically calculate anticipated entrapment efficiency in the liposome. Dibucaine equilibrium solubility in pH 5.6 and pH 9.0 hydration media was determined in the present work and found to be 8.6 and 1.4 mg/mL, respectively. These solubilities predict entrapment efficiencies of 23.6% and 87.6% for hydration media pH 5.6 and pH 9 respectively. The corresponding experimentally determined values for the entrapment efficiency were close to calculated values (26.97% ± 1.5 and 78.82% ± 0.55 for pH 5.6 and pH 9.0, respectively).

Other key issues for the successful preparation of dibucaine liposomes proved to be: composition of organic solvent mixture (chloroform/methanol 7:3 v/v) used to dissolve the lipids and the drug, temperature selected for evaporating the organic solvent to yield the lipid film (43°C which is ~10°C higher than the gel-to-fluid crystalline transition temperature of the phospholipid used), lipid film hydration time, mode of agitation during hydration, and allowing sufficient time for maturation of the liposomes (overnight at 4°C). These factors were reported to be important determinants for the successful preparations of liposomes (14–16).

Increasing cholesterol : phosphatidylcholine molar ratio only slightly increased dibucaine entrapment in liposomes prepared in phosphate buffer pH 5.6 (Figure 3). The effect was more pronounced for liposomes prepared in bicarbonate buffer pH 9.0 (Figure 3). In both media there was a trend towards maximum entrapment efficiency at a lipid : cholesterol molar ratio of 7:6 beyond which the entrapment efficiency tended to decrease.

Increased entrapment efficiency attributed to cholesterol may be due to increased stability of the liposomal membrane during hydration. Cholesterol increases rigidity of the bilayer (17) and decreases permeability of liposomes (18). However, increasing the cholesterol beyond a certain concentration can disrupt the regular linear structure of the liposomal membrane, thereby reducing the entrapment efficiency as reported by other researchers (15, 18).

Stearylamine was used to impart a positive charge to liposomes prepared in phosphate buffer (pH 5.6) medium. A decrease in entrapment efficiency (from 26.97% ± 1.5% to 20.57% ± 2.23%) resulted from the incorporation of stearylamine.
Effect of various formulation variables on the encapsulation of dibucaine compared to neutral liposomes prepared under the same conditions (Figure 3).

Comparable results were obtained with bicarbonate buffer (pH 9.0) medium. The corresponding entrapment efficiency values recorded were 79.59% ± 3.12% for neutral liposomes and 59.01% ± 3.39% for positively charged ones (Figure 3).

Dicetylphosphate was used to impart a negative charge to liposomes prepared in phosphate buffer pH 5.6. An increase in entrapment efficiency (from 26.97% ± 1.5% to 40.45% ± 3.42%) resulted from the incorporation of dicetylphosphate compared to neutral liposomes prepared under the same conditions (Figure 3).

Comparable results were obtained with bicarbonate pH 9.0. The corresponding entrapment efficiency values recorded were 79.59% ± 3.12% for neutral liposomes and 91.76% ± 3.47% for negatively charged ones (Figure 3).

The decrease in entrapment efficiency observed with positively charged liposomes could be related to slight electrostatic repulsive force occurring between the partial positive center on the drug molecules acquired from the hydration medium and stearylamine. This repulsion may account for the lower entrapment efficiency values when compared to the negatively charged liposomes where an electrostatic attraction could lead to higher entrapment efficiency.

Apart from attractive forces holding drug molecules, the inclusion of negatively charged molecules has been reported to increase the distance between adjoining lamellar bilayers due to the electrostatic repulsion between adjacent bilayers, resulting in entrapment of larger amounts of aqueous solutions (19). This could be a factor promoting entrapment of a hydrophilic drug or a charged hydrophobic drug.

Electron photomicrograph obtained for some of the dibucaine liposomal dispersions at different magnifications, using two staining methods, is shown in Figure 4. The multilamellar nature of the prepared liposomes is evident.

Corresponding median vesicle diameter data are given in Table 2. The computed median vesicle diameter (~ 1.5 µm) is within the reported vesicle size for multilamellar liposomes (11).

Cumulative percent dibucaine release values over 12 h determined for eight of the liposomal dis-
persions are shown in Figure 5 a and b. Compared to the control data, dibucaine release from liposomes is prolonged.

Doubling the cholesterol ratio (D₆ pH₉ compared to D₃ pH₉ and D₆ pH₅ compared to D₃ pH₅) decreased the release rate. Positively charged liposomes [(D₆ pH₉ (+) and D₆ pH₅ (+)] exhibited the fastest release among liposomal dispersions.

Judging by the amount of dissolved dibucaine, ~ 10–30% of the drug is released at a relatively rapid rate during the first 2 h followed by slower release rates over the next 10 h. The initial rapid phase of drug release is less evident in case of negatively charged liposomes.

Looking into the kinetics of dibucaine release from the liposomes, the release data from time zero to 12 h appear to best fit the Higuchi’s diffusion model based on the magnitude of correlation coefficient obtained for zero order, first order and Higuchi’s model (20). Similarly, looking into the kinetics of drug release during the relatively slow phase of release (2–12 h), the highest correlation coefficients were obtained when the data were fitted to the Higuchi model (21).

The initial rapid phase of release is evident. The percent drug released during this initial phase was estimated from intercepts of the Higuchi’s plots for release data from 2–12 h. Intercept values ranged from 10.40% ± 1.42% to 24.26% ± 2.63% approached zero for the negatively charged liposomes.

### Table 1. Composition of dibucaine liposomes prepared by the lipid film hydration method.

<table>
<thead>
<tr>
<th>Code</th>
<th>pH of hydration medium</th>
<th>Phosphatidylcholine/cholesterol/charge imparting agent molar ratio</th>
<th>Charge imparting agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>D₀ pH₉</td>
<td>9</td>
<td>7/0/0</td>
<td>–</td>
</tr>
<tr>
<td>D₃ pH₉</td>
<td>9</td>
<td>7/3/0</td>
<td>–</td>
</tr>
<tr>
<td>D₆ pH₉</td>
<td>9</td>
<td>7/6/0</td>
<td>–</td>
</tr>
<tr>
<td>D₆ pH₅</td>
<td>5.6</td>
<td>7/0/0</td>
<td>–</td>
</tr>
<tr>
<td>D₇ pH₅</td>
<td>5.6</td>
<td>7/3/0</td>
<td>–</td>
</tr>
<tr>
<td>D₆ pH₅(−)</td>
<td>5.6</td>
<td>7/6/0</td>
<td>–</td>
</tr>
<tr>
<td>D₆ pH₉(−)</td>
<td>5.6</td>
<td>7/6/1</td>
<td>Dicetylphosphate</td>
</tr>
<tr>
<td>D₆ pH₉(+)</td>
<td>5.6</td>
<td>7/6/1</td>
<td>Stearylamine</td>
</tr>
</tbody>
</table>

### Table 2. Mean vesicle diameter of different dibucaine liposomal dispersions. (Each result represents the mean ± standard deviation of three independent experiments)

<table>
<thead>
<tr>
<th>Code</th>
<th>Vesicle diameter (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dₖ pH₅</td>
<td>1.5 ± 0.06</td>
</tr>
<tr>
<td>Dₖ pH₅(−)</td>
<td>1.6 ± 0.05</td>
</tr>
<tr>
<td>Dₖ pH₅(+)</td>
<td>1.2 ± 0.14</td>
</tr>
<tr>
<td>D₆ pH₉</td>
<td>1.6 ± 0.09</td>
</tr>
<tr>
<td>D₆ pH₉(−)</td>
<td>1.7 ± 0.12</td>
</tr>
<tr>
<td>D₆ pH₉(+)</td>
<td>1.3 ± 0.16</td>
</tr>
</tbody>
</table>

### Table 3. Visual stability rating of dibucaine liposomal dispersions after storage at 4°C for 12 months.

<table>
<thead>
<tr>
<th>Code</th>
<th>Physical stability*</th>
<th>Optical microscopy examination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dₖ pH₅</td>
<td>Separated (4)**</td>
<td>Intact vesicles observed no signs of coalescence</td>
</tr>
<tr>
<td>Dₖ pH₅(−)</td>
<td>Stable (8)**</td>
<td></td>
</tr>
<tr>
<td>Dₖ pH₅(+)</td>
<td>Stable (8)**</td>
<td></td>
</tr>
<tr>
<td>D₆ pH₉</td>
<td>Separated (6)**</td>
<td></td>
</tr>
<tr>
<td>D₆ pH₉(−)</td>
<td>Stable (8)**</td>
<td></td>
</tr>
<tr>
<td>D₆ pH₉(+)</td>
<td>Separated (7)**</td>
<td></td>
</tr>
</tbody>
</table>

* Visual physical stability was recorded 12 months after preparation.
** Visual Stability rating for disperse system products (12).
Drug release profiles from multilamellar liposomes appearing in the literature characteristically show an initial fast drug loss followed by slower rates of drug loss (22). The initial fast rate of release is commonly ascribed to drug detachment from liposomal surface while the latter slow release results from sustained drug release from the inner lamellae. The release profiles generated in this paper for dibucaine base multilamellar liposomes bear the same features. Values calculated for Higuchi’s plot intercept (percent released at zero time) were taken as a measure of percent dibucaine associated with liposome surface and released during the initial fast drug loss. It was also assumed in another study (23) involving
liposome entrapped ampicillin that at time zero of the release run, the system contains both encapsulated and unencapsulated drug, and release could be described as resulting from a series of parallel first order processes.

Zero, first order and Higuchi’s equations were applied to in vitro release results. Correlation coefficient values were high in all cases but the best fitting model was found to be the Higuchi’s model suggesting that drug transport out of the liposomes was driven mainly by a diffusion-controlled mechanism. Negatively charged liposomes exhibited low release rates. The negative charge imparting agent conjugated with the phospholipids bilayer structure presumably attracts the ionized part of dibucaine decreasing its release (19).

One of the liposomal concentrates included in the release study was examined using the electron microscope before and after the release run to detect effects, if any, of release study conditions on the integrity of the liposomes (Figure 6 a and b). No evidence of large scale lysis or coalescence of the vesicles could be found. The retention of sealed vesicular structures after the release run is evident.

Six of the liposome dispersions were stored at room temperature for 12 months. Results of visual stability rating of these liposomes after 12 months storage are given in Table 3; the rating system (12) used is shown in Table 4. The information gained is that the charged liposomes are more stable in terms of homogeneity and physical stability compared to uncharged liposomes. Concerning possible coalescence and aggregation during storage, no coalescence was observed microscopically in the six dispersions examined.

Apart from visual stability rating, negatively charged liposome concentrates were stored at 4°C for 4 weeks. Results of in vitro release profiles of these liposomes over 4 week study period are given in Figure 7 a and b. The results indicate progressive increase in release rate particularly evident by the fourth week of storage.

Comparing release profiles before and during storage indicated a rise in the percent drug released during the initial faster phase of release (0–2 h) as evi-

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>No visual separation, completely homogenous</td>
</tr>
<tr>
<td>8</td>
<td>No visual separation, virtually homogenous</td>
</tr>
<tr>
<td>7</td>
<td>Very indistinct separation, no clear layer at bottom or top</td>
</tr>
<tr>
<td>6</td>
<td>Indistinct separation, no clear layer at bottom or top</td>
</tr>
<tr>
<td>5</td>
<td>Distinct separation, no clear layer at bottom or top</td>
</tr>
<tr>
<td>4</td>
<td>Homogenous top or bottom layer, clear layer at bottom or top</td>
</tr>
<tr>
<td>3</td>
<td>Distinct separation, clear layer at bottom or top with no coalescence</td>
</tr>
<tr>
<td>2</td>
<td>Distinct separation with slight coalescence</td>
</tr>
<tr>
<td>1</td>
<td>Distinct separation with strong coalescence</td>
</tr>
<tr>
<td>0</td>
<td>Complete separation and complete coalescence</td>
</tr>
</tbody>
</table>

Figure 6. Electron photomicrograph of dibucaine liposomal dispersion (DpH5): (a) before the release run. (b) after terminating the release run.
denced by the gradual increase in Higuchi’s plot intercept as a function of storage time (Figure 8 a and b).

Possible drug leakage out of liposomes during storage of liposome concentrate was monitored by recording changes in release rates of negatively charged drug-loaded liposomes as a function of storage time. In this respect, it has been suggested that in vitro release studies in liposome research are not meant to mimic in vivo conditions but may be considered a measure of instability. Higuchi’s plot intercept, considered as a measure of unentrapped drug associated with the liposomes, was found to increase as a function of storage time (Figure 8 a and b) suggesting progressive drug leakage out of liposomes stored at 4°C in the form of aqueous liposome dispersions.

Figure 7. Release stability profiles at 32°C as a function of storage time at 4°C (a) of dibucaine liposomal dispersion (D2pH5 (→)). (b) of dibucaine liposomal dispersion (D2pH9 (→)). (Each result represents the mean of three independent experiments)
CONCLUSION

The results obtained indicated the successful preparation of dibucaine base multilamellar liposomes with the potential for sustained drug release. The highest entrapment efficiency was achieved with negatively charged liposomes, prepared with a lipid:cholesterol:dicetylphosphate molar ratio of 7:6:1, using alkaline medium of pH 9 as a hydration medium for the lipid film.

The release data also pointed to the negatively charged liposomes as having a more sustained and prolonged drug release than the other prepared liposomes and having less burst effect (initial fast release phase) attributed to drug associated with liposome surface.

The stability results indicated that charged liposomes when stored at room temperature as diluted aqueous dispersions are more stable in terms of homogeneity and physical stability compared to uncharged liposomes.

Further investigations should be developed to increase stability of dibucaine multilamellar vesicles so as to be a successful topical local anesthetic delivery system. Such investigations may include coating the liposomal surface by low molecular weight polymers, incorporating the liposomal vesicles in gel dosage form or lyophilization of dibucaine liposomes using a suitable cryoprotectant such as trehalose.

REFERENCES


Received: 3.08.2005