References


Liposomal Drug delivery of Zidovudine and it's Evaluation


transfer to the nasal epithelium of patients with cystic fibrosis. Gene Ther. 4: 199-209.


59) Lars Ingebrigtsen and Martin Brandl. (2002). Determination of the Size Distribution of Liposomes by SEC Fractionation, and PCS Analysis and Enzymatic Assay of Lipid Content. AAPS.


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[265]

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Liposomal Drug delivery of Zidovudine and It's Evaluation


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Liposomal Drug delivery of Zidovudine and it's Evaluation
Liposomal Drug delivery of Zidovudine and it's Evaluation


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**Liposomal Drug delivery of Zidovudine and It’s Evaluation**


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Liposomal Drug delivery of Zidovudine and it’s Evaluation


Liposomal Drug delivery of Zidovudine and it's Evaluation
List of Publications

PAPERS PUBLISHED:

1. Cyanocobalamine resinate and its stability studies Published in Asian Journal of Chemistry.
2. Formulation and evaluation of Buccal Patches of Ketrolac tromethamine published in Biosciences, Biotechnology, research Asia.
5. Nanoparticles as drug delivery system published in The Pharmacist bi annual issue
6. Applications of liposomes: A Review published in The Pharmacist bi annual issue

PAPERS ACCEPTED:

1. In-vitro Release Behavior and Stability of Liposomal Formulation of Zidovudine accepted in Advances in Pharmacology and Toxicology
2. In-vitro and In-vivo Release of Zidovudine from Different Liposomal Gel in Rabbit Model accepted in International Journal of Pharmacology and Biological Sciences.
3. Effects of pH, Salt, Temperature on conventional liposomes size enlargement analyzed by optical microscope accepted in Indian Journal of Applied Life Sciences.
4. Natural Soya Lecithin as a Drug Delivery-A Review accepted in Plant Archives.

PAPERS COMMUNICATED:

To,
Prof/Dr. S. Palani
Inst. of Pharmacy
Banaras Hindu Univ.
Varanasi - 221005

Dear Dr. Palani,

I am pleased to inform you that your paper, “In Vitro release of Zidovudine” by Sharma & Prakash, has been accepted for publication in the Journal “Advances in Pharmacology and Toxicology.”

It will be published in Vol. 8, No. 3, of 2007.

With best wishes.

Yours sincerely,

Ram Prakash

(Ram Prakash)

Invitro Release Behavior And Stability Of Liposomal Formulation Of Zidovudine

P.K.SHRMA, S.PALANI*, R. IRCHHIAYA
Institute of Pharmacy, Bundelkhand University, Jhansi-284003

ABSTRACT:

The objective of the present study was to develop the release kinetics of liposome encapsulated material in the presence of phospholipids. To determine the factors influencing encapsulation of zidovudine in liposomes and to optimize the encapsulation parameter. Zidovudine was encapsulated in multilamellar liposomes, prepared using Phosphotidyl choline and cholesterol. The effects of method of preparation, type of vesicle formed, charge of the vesicle by using zeta sizer, Concentration of cholesterol in encapsulation of zidovudine in liposomes were investigated. Also releases of zidovudine under various conditions like temperature, Stirring speed and invitro drug release from liposomes was studied using a dialysis method by using the dialysis bag.

Keywords: Liposomes, Zidovudine, Liposomal Gel, Topical application

INTRODUCTION:

Liposomes, after three decades of research are still gaining knowledge with special emphasis more recently on their use as drug carrier systems (D.D.Lasic, 1993). Liposomes have been successfully used as delivery vehicles to improve therapeutic efficacy and reduce toxic effects (G.Gregorladis, 1988). For therapeutic purposes they must be loaded with drug substance. This is more essential achieved with amphiphile molecules like phospholipids and cholesterol as they have a tendency to be incorporated in the liposomal membrane. In contrast, hydrophilic molecules must be encapsulated in the aqueous interior, which in general, cannot easily be performed in an efficient manner (M.J.Ostro1987). A variety of liposomes preparations were introduced and they have to fulfill the basic requirements (a) Produced liposomes should be homogeneous (b) Efficient of encapsulation of hydrophilic drugs. Where as thin film hydration technique achieve high efficiency and easy method to produce in the laboratories (R.L. Jullano et al). The concept of this project was fairly to entrap hydrophilic drugs like zidovudine. The ratio of volume inside the liposomes compared to the total aqueous volume of the preparation is encapsulation efficiency. The increasing lipid concentration more liposomes per unit volume of the preparation are formed. When phospholipid dispersed in aqueous medium at certain concentrations results in highly viscous dispersions up to semisolid consistency. So, the phospholipid, cholesterol and hydration times were changed to achieve maximum entrapment efficiency.

Zidovudine, the first anti HIV compound approved for clinical use is widely used for the treatment of AIDS either alone or combination with other antiviral agents. However, the main limitation to therapeutic effectiveness of zidovudine its dose dependant hematological
toxicity, low therapeutic index, short biological half life and poor bioavailability (Kleburtz et al). Zidovudine available in the market as conventional tablet form. After oral administration, it is rapidly absorbed from GIT exhibiting a peak plasma concentration of 1.2μg/ml at0.8 hours(R.W.Klecker et al). In the systemic circulation, it is first converted to Azidothymidine triphosphate, which is pharmacologically active and prevents the replication of the HIV virus. The biological half-life of zidovudine is 4 hours. To maintain the constant therapeutic drug levels and to target the virus an adequate zero order delivery of zidovudine required. Therefore a simple method was choosed to study the release kinetics of liposomal encapsulated material such as zidovudine.

PREPARATION OF LIPOSOMES:

Multilamellar liposomes were prepared using the thin film hydration method (Bangham et al). Accurately weighed quantity of drug, Phosphotidyl choline, Cholesterol. Phosphotidyl choline, Cholesterol in the ratio of 200mg: 80mg were dissolved in two parts of chloroform and one part of methanol mixture in 250 ml spear shaped flasks. The chloroform and methanol mixture was evaporated at 45°C under reduced pressure (600psi) at 150rpm using Rota evaporator. After the chloroform and methanol was completely evaporated. The flask was kept overnight under vacuum pressure to remove residual solvent. The thin film was hydrated using drug-containing saline for 2 hours until vesiculation was completed.

Estimation of Entrapped Drug in Liposomes

Zidovudine entrapped within the liposomes was estimated after removing the unentrapped drug. The unentrapped drug was separated from the liposomes by subjecting the dispersion to centrifugation in a cooling centrifuge (Remi Equipments, Mumbai, India) at 15000 rpm at a temperature of −4°C for 30 minutes, whereupon the pellets of liposomes and the supernatant containing free drug were obtained. The liposome pellets were washed again with distilled water to remove any unentrapped drug by centrifugation(Kulkarni et al). The combined supernatant was analyzed for the drug content after suitable dilution with saline solution by measuring absorbance at 267 nm using Shimadzu U-V 1700(U-V Spectrophotometer). The percentage drug entrapment in the liposomes was calculated from the difference between the initial drug added and the drug detected in the supernatant. There are two methods to find out the entrapment efficiency, the first one is by using the methanol and second one is by using detergent like TritonX-100.

The amount of drug exactly present within the liposomes was also analyzed by dissolving the liposomes in methanol to countercheck the percentage drug entrapment and to arrive at a mass balance. The analysis of drug in liposomes was carried out using the empty liposomes dissolved in methanol as blank in order to nullify the interference of the excipients.

Encapsulation efficiency:

The pellets obtained from centrifugation were resuspended in 0.9% w/v saline solution. The influence of detergent on liposomes solubilisation was determined by dissolving
300μl of liposome suspension with 0.5%, 1%, 2%, 3%, 4%, 5%, and 6% with different concentrations to find out the exact concentration of Triton X-100 detergent. The detergent solution was then gently heated for 5 minutes. The clear solution was quantitatively analyzed for zidovudine by spectrophotometry at 267nm.

**Dialysis bag method:**

Zidovudine release from liposomes was determined by using the dialysis bag. The dialysis bag membrane is permeable to release medium and it can allow the drug such as zidovudine. Before using the dialysis bag was soaked in water for around four hours to remove the contaminants and debris in the membrane. A 4.0 ml of zidovudine liposomal suspension and empty liposomal suspension separately was taken in to beakers containing 100ml saline solution was the drug release medium. The medium temperature was maintained at 37°C prior to use, in order to deearate the medium, the stirring speed and temperatures were maintained at 100± 10 rpm and 37 ± 0.5°C respectively. An aliquot of 2ml sample was withdrawn at different time intervals for the determination of zidovudine concentration by U-V spectroscopy at 267 nm. At the same time, same volume of water was replaced to maintain the sink condition.

The empty liposomes was taken to found that the absence of zidovudine, no λ max at 267 nm was measured in solvent, which indicates that the liposomes constituents do not interfere with the measurement of zidovudine concentration. The above release test was to conform the interference of phospholipids or other substances.

**Optical microscopy:**

Zidovudine liposomal dispersion was examined by optical microscope (Magnus-DLX-DX, Olympus Labs) before and after the release of drugs from liposomes.

**Transmission electron microscopy:**

Zidovudine liposomal dispersion was examined by Transmission electron microscope to find out the presence of spherical vesicles and type of vesicles.

**Determination of zeta potential:**

The zeta potential is the overall charge a particle acquires in a particular medium. Both mean particle size and charge of the particles can be measured on a Malvern Zetasizer. The zeta potential of a liposome preparation can help to predict the fate of the liposomes in vivo and invitro. Any subsequent modification of the liposome surface can also be monitored by measurement of the zeta potential(D.J.Crommelin et al).

**Stability of the liposomal dispersions:**

The drug loaded liposomal dispersions were stored at 4°C, 25°C and 37°C. The liposomes were observed under microscope for the size and appearance at the end of every week. The stability test was conducted for 4 weeks to find out the drug stability in the vesicles.
RESULTS AND DISCUSSION:

The ratio of the Phosphotidyl choline, cholesterol, hydration times and rotation time of the flask were varied to get the maximum entrapment of zidovudine a hydrophilic drug.

Table-1 : Composition, Hydration time, Mean particle size

<table>
<thead>
<tr>
<th>S. No.</th>
<th>PC: CH (mg)</th>
<th>Drug (mg)</th>
<th>Hydration time</th>
<th>Rotation time of the flask</th>
<th>EE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>200:200</td>
<td>10</td>
<td>02</td>
<td>50</td>
<td>35±0.8</td>
</tr>
<tr>
<td>2.</td>
<td>200:100</td>
<td>10</td>
<td>04</td>
<td>100</td>
<td>42±1.2</td>
</tr>
<tr>
<td>3.</td>
<td>200:80</td>
<td>10</td>
<td>06</td>
<td>150</td>
<td>53±1.0</td>
</tr>
<tr>
<td>4.</td>
<td>200:80</td>
<td>10</td>
<td>08</td>
<td>150</td>
<td>59±1.6</td>
</tr>
<tr>
<td>5.</td>
<td>200:60</td>
<td>10</td>
<td>08</td>
<td>150</td>
<td>51±1.8</td>
</tr>
</tbody>
</table>

PC- Phosphotidyl Choline
CH-Cholesterol
EE-Entrapment Efficiency
(n=3)

The data of the table-1 showed that the thick film of lipids which gave liposomes with less entrapment of zidovudine. The thin film showed better entrapment than thick film i.e. 35±0.8% compared to the entrapment 59±1.6% obtained in liposomes prepared by thin film. The effects of the formulation variables, the lipid phase composition PC/CH and hydration condition on drug entrapment efficiency and mean particle size of liposome vesicles. Decreasing amount of cholesterol in the lipid phase and increasing the drug concentration, the entrapment efficiency of zidovudine in to liposomes increased(G.Gregoriadis et al). The drug substance has a slight affinity for the lipid phase. The encapsulation of the zidovudine in the liposomes is related to the overall volume of aqueous phase encapsulated during liposomes formulations. Triton-X 100 is used to separate drug from the liposomes, 5% is suitable. Below and above 5% showed less drug concentration. So, a 5% triton-X 100 concentrations is suitable to find out the drug entrapment in the liposomes. The prepared liposomes are of similar size, with the mean diameter of 1100nm and the charge of the liposomes was observed that the particles are negatively charged, the range is -22.2. Transmission electron microscope revealed the presence of spherical vesicles (Liposomes). The DSC studies were conducted to obtain evidences of the drug entrapment by the liposomes. The transition temperature was increased, so it confirms the drug entrapment. The phase transition temperature can give good clues about liposomal stability, permeability and whether a drug is entrapped in the bilayered or in the aqueous compartment (M.Weiner et al). The drug release of the conventional liposome formulations is present in the table-2 and fig-1. It was found that 98.64% of encapsulated drug was released during a period of 8 hours, in 37°C temperature. The hydration condition, an increase of hydration time, led to a slight increase of drug entrapment showed in table-1.
<table>
<thead>
<tr>
<th>S. No</th>
<th>Time In hours</th>
<th>% Drug release from conventional liposomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2.</td>
<td>0.5</td>
<td>12.5</td>
</tr>
<tr>
<td>3.</td>
<td>1</td>
<td>21.45</td>
</tr>
<tr>
<td>4.</td>
<td>1.5</td>
<td>29.56</td>
</tr>
<tr>
<td>5.</td>
<td>2</td>
<td>38.2</td>
</tr>
<tr>
<td>6.</td>
<td>2.5</td>
<td>50.74</td>
</tr>
<tr>
<td>7.</td>
<td>3</td>
<td>62.48</td>
</tr>
<tr>
<td>8.</td>
<td>3.5</td>
<td>71.25</td>
</tr>
<tr>
<td>9.</td>
<td>4</td>
<td>84.5</td>
</tr>
<tr>
<td>10.</td>
<td>6</td>
<td>92.01</td>
</tr>
<tr>
<td>11.</td>
<td>8</td>
<td>98.23</td>
</tr>
<tr>
<td>12.</td>
<td>10</td>
<td>98.56</td>
</tr>
<tr>
<td>13.</td>
<td>12</td>
<td>98.99</td>
</tr>
<tr>
<td>14.</td>
<td>14</td>
<td>98.74</td>
</tr>
<tr>
<td>15.</td>
<td>16</td>
<td>98.01</td>
</tr>
<tr>
<td>16.</td>
<td>18</td>
<td>99.01</td>
</tr>
<tr>
<td>17.</td>
<td>20</td>
<td>98.38</td>
</tr>
<tr>
<td>18.</td>
<td>22</td>
<td>98.12</td>
</tr>
<tr>
<td>19.</td>
<td>24</td>
<td>98.64</td>
</tr>
</tbody>
</table>

**Fig-1** Percentage drug release from conventional liposomes
The temperature of the release medium may alter the viscosity of the liposomal suspension. Generally, the human body temperature is 37°C. However, in the clinical cases, such as fever, the variation of temperature can change the drug release when liposomes are sensitive to the temperature (D. Papahadjopoulos et al). The fig-2 shows that increasing temperature of the medium increases zidovudine release from the conventional liposomes. The release studies were conducted for 2 hours to find the behavior of liposomes at different temperature. Stirring speed kept constant at 100rpm. The temperature of the medium has no significant effects on zidovudine release from liposomes in the range 37-39°C. Which means that drug release from the liposomes in non-temperature sensitive invitro at body temperature. But with increased temperature, the zidovudine release rate increased. The effect because of the rising temperature increases the value of diffusion.

**Table-3** : Zidovudine release from conventional liposomes at 25°C, 37°C, 39°C, 50°C

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Time in minutes</th>
<th>% Drug released at 25°C</th>
<th>% Drug released at 37°C</th>
<th>% Drug released at 39°C</th>
<th>% Drug released at 50°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2.</td>
<td>10</td>
<td>2.53</td>
<td>6.24</td>
<td>7.51</td>
<td>18.02</td>
</tr>
<tr>
<td>3.</td>
<td>20</td>
<td>7.42</td>
<td>16.03</td>
<td>18.62</td>
<td>26.32</td>
</tr>
<tr>
<td>4.</td>
<td>30</td>
<td>10.54</td>
<td>21.54</td>
<td>22.67</td>
<td>35.45</td>
</tr>
<tr>
<td>5.</td>
<td>40</td>
<td>14.65</td>
<td>23.99</td>
<td>25.87</td>
<td>43.87</td>
</tr>
<tr>
<td>6.</td>
<td>50</td>
<td>16.87</td>
<td>24.87</td>
<td>28.36</td>
<td>47.85</td>
</tr>
<tr>
<td>7.</td>
<td>60</td>
<td>20.41</td>
<td>27.8</td>
<td>30.84</td>
<td>53.01</td>
</tr>
<tr>
<td>8.</td>
<td>90</td>
<td>25.28</td>
<td>32.69</td>
<td>36.44</td>
<td>61.22</td>
</tr>
<tr>
<td>9.</td>
<td>120</td>
<td>29.98</td>
<td>38.54</td>
<td>42.57</td>
<td>72.42</td>
</tr>
</tbody>
</table>

**Figure-2** : Zidovudine release from conventional liposomes at 25°C, 37°C, 39°C, 50°C
Effect of stirring speed on the release of drugs from liposomes:

The drug release test is conducted using a beaker with a magnetic stirrer. However, for the liposomes, there is no detailed test in the official books. The stirring speed is varied based on the characteristic of drugs and the testing devices. The effect of stirring speed on the zidovudine release from liposomes is shown in fig-3 and table-4. It was shown in figure and table that increased stirring speed increase the rate of drug release. In addition with an increased stirring speed, the equilibrium time for drug release from conventional liposome decreased gradually. If a constant speed applied to the release device, the diffusion layer becomes thinner and drug release rate become larger. An increase in speed leads to reduction in the thickness of the diffusion layer. When the stirring speed 200 or more than two hundred and the dialysis bag may also be destroyed. Therefore an optimal stirring speed was 100rpm.

Table-3 : Zidovudine release from conventional liposomes at 25°C, 37°C, 39°C, 50°C

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Time in minutes</th>
<th>% Drug released at 25°C</th>
<th>% Drug released at 37°C</th>
<th>% Drug released at 39°C</th>
<th>% Drug released at 50°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2.</td>
<td>1</td>
<td>2.53</td>
<td>6.26</td>
<td>10.21</td>
<td>24</td>
</tr>
<tr>
<td>3.</td>
<td>2</td>
<td>5.64</td>
<td>16.73</td>
<td>18.45</td>
<td>33.3</td>
</tr>
<tr>
<td>4.</td>
<td>3</td>
<td>9.68</td>
<td>21.52</td>
<td>22.48</td>
<td>38.7</td>
</tr>
<tr>
<td>5.</td>
<td>4</td>
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<td>23.79</td>
<td>26.79</td>
<td>43.1</td>
</tr>
<tr>
<td>6.</td>
<td>5</td>
<td>15.64</td>
<td>24.89</td>
<td>29.62</td>
<td>45.8</td>
</tr>
<tr>
<td>7.</td>
<td>6</td>
<td>18.72</td>
<td>27.26</td>
<td>32.14</td>
<td>49.4</td>
</tr>
<tr>
<td>8.</td>
<td>7</td>
<td>22.02</td>
<td>31.62</td>
<td>36.54</td>
<td>54.8</td>
</tr>
<tr>
<td>9.</td>
<td>8</td>
<td>26.58</td>
<td>38.54</td>
<td>45.23</td>
<td>62.9</td>
</tr>
</tbody>
</table>

Figure-3 : Zidovudine release from conventional liposomes at 25°C, 37°C, 39°C, 50°C
REFERENCES:


- G.Gregoriadis, C.Davis, Stability of liposomes in vivo and invitro is promoted by their cholesterol content and the present of blood cells, Biochim.Biophys. Res. commun. 89 (1979) 287-293.


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To: "navneet garud" <navneet_garud@rediffmail.com>

Subject: Acceptance of Paper

Date: Tue, 07 Aug 2007 13:46:27 IST

Note: To help protect your privacy, images from this message have been blocked. View images | What is

Dear Dr. S. Palani,

I am pleased to inform you that your research paper <Invitro release of-------rabbit model> has been accepted for publication in INTERNATIONAL JOURNAL OF PHARMACOLOGY AND BIOLOGICAL SCIENCES. It will appear in VOL. 2 NO. 1 2008. Thanks for your interest.
ABSTRACT:

The objective of the present study was to develop a liposomal gel for the topical application of Zidovudine an anti HIV drug and capable to efficiently deliver entrapped drug during an extended period of time. Zidovudine was entrapped in the liposomes composed of Phosphotidyl Choline and cholesterol in the ratio of 200mg: 80mg and prepared by thin film hydration method. Liposomes preparation was characterized and compared for particle size mean dispersion, entrapment efficiency, DSC, Zeta potential and tested for in-vivo release in rabbits. In-vitro release studies also performed to compare the in-vitro and in-vivo release of Zidovudine. To achieve Application viscosity of liposomes and to further improve their stability than conventional liposomes. The liposomes prepared by thin film hydration method was incorporated in the bioadhesive gels like 2%, 3% of Carbopol 940, 2%3% HPMC K4 100. All the performed experiments confirm the applicability of liposomes as novel drug delivery carrier system for the topical application of Zidovudine.

Keywords: Liposomes, Zidovudine, Gel, Topical application.

INTRODUCTION:

Topical delivery systems for systemic administration of several drugs have been extensively investigated over last decade (M.Schafer-Korting et al). The topical dosage form attempts are being made to utilize drug carriers that ensure adequate of the drug with in the skin in order to enhance local and minimize side effects (F.P.Bonina et al). Advantage of the topical route of drug administration (a) avoidance of first pass metabolism (b) reduction in the incidence (c) Severity of GIT effects (d) Easiness of application (e) Complete privacy of the therapy for the patients. Liposomes are becoming increasingly important as carriers of biologically important molecules in living system. (Tyrell et al., 1976; Papahadjiopoulos, 1978; Gregoriadis,1980; Roerdink et al., 1981). The application of liposomes as drug carrier on the skin surface has been proven to efficient in the delivery of liposomes entrapped drugs to and in to the skin. Liposomes applied on the skin, may act as a solubilizing matrix for poorly soluble drugs and as well as local depot for sustained drugs release (M.J.Ostro1987). Summarily, topical liposome formulation could be more effective than conventional formulations. Topical application of gel form, hydrophilic polymers are considered to be suitable thickening agents. The polymer, which forms the gel matrix, could influence the stability as well as the release rate of incorporated drugs.

Carbopol polymers, a well-known polymer acrylic acid cross-linked with polyalkenyl ethers. Each particles of Carbopol can be viewed as a network structure of polymer chains
In addition to its hydrophilic nature, its cross-linked structure and it essentially insoluble in water makes Carbopol a potential candidate for use in controlled release drug delivery system (A.T. Florence et al). Hydrophilic polymer gel matrix systems are widely used in controlled drug delivery because of their flexibility to obtain a desirable drug release profile, cost effectiveness, and broad U.S. Food and Drug Administration acceptance. Hydroxypropyl methyl cellulose (HPMC), which is commonly used in hydrophilic matrix drug delivery systems, is mixed alkyl hydroxyl alkyl cellulose ether containing methoxyl and hydroxypropyl groups. The hydration rate of HPMC depends on the nature of these substituents, such as the molecular structure and the degree of substitution. Specifically, the hydration rate of HPMC increases with an increase in the hydroxypropyl content. The solubility of HPMC is pH independent.

Previous studies investigated that application of liposomes containing drug is possible to extend the release rate of water-soluble drugs. Continuing that research, here we report the development of a bioadhesive liposomal gel containing Zidovudine for the topical applications.

**MATERIALS AND METHODS:**

**Materials:**

The materials used in the study were Zidovudine a gifted sample of Alkem Laboratories Ltd, Raigad. Phosphatidyl choline, Qualigens, Mumbai. Cholesterol, Qualigens, Mumbai. Chloroform, Merck, Mumbai. Methanol, Qualigens, Mumbai. were Purchased. All in the study, other ingredients used were of analytical grade.

**Methods:**

**Preparation of Liposomes:**

Multilamellar liposomes were prepared by using thin film hydration method (Bangham et al)) Accurately weighed quantity of Drug, Phosphatidyl Choline, Cholesterol was taken. Phosphatidyl Choline, Cholesterol in the ratio of 200mg: 80mg were dissolved in two parts of chloroform and one part of methanol mixture in 250 ml spear shaped flask. The chloroform and methanol mixture was evaporated at 45°C under reduced pressure (600psi) at 150rpm using Rota evaporator. After the chloroform and methanol was completely evaporated. The flask was kept overnight under vacuum pressure to remove residual solvent. The thin film was hydrated using drug-containing 0.9%w/v Saline solution for 8 hours until vesiculation was completed.

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Zidovudine liposomal dispersion was examined by Transmission electron microscope to find out the presence of spherical vesicles and type of vesicles.

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The zeta potential is the overall charge a particle acquires in a particular medium. Both mean particle size and charge of the particles can be measured on a Malvern Zetasizer. The zeta potential of a liposome preparation can help to predict the fate of the liposomes in vivo and invitro. Any subsequent modification of the liposome surface can also be monitored by measurement of the zeta potential (D.J.Crommelin et al).

In-vitro release studies:

Studies of drug release from liposomal gel formulations were performed using the modified Franz diffusion cell (Vertical type). Drug release from liposomes was studied by using a dialysis method (R.Peschka et al). Before using the membrane the dialysis membrane was soaked in distilled water at room temperature for 2 hours to remove the preservatives, followed by rinsing thoroughly in distilled water. The dialysis membrane was cut in to 2.5cm x 2.5cm². The dialysis membrane was cut mounted between donor and receptor compartments. The diffusion area was 1.5cm± 0.01cm2. The receptor compartment (in contact with receptor membrane) was filled with 70 ml of saline solution and the receptor compartment temperature was maintained 37°C ± 0.5°C. Then donor compartment was then covered with aluminium foil to prevent evaporation of sample. At predetermined time intervals, 2.0ml of samples were withdrawn from the receptor compartment and for subsequent analysis of Zidovudine by spectrometric method at 267 nm.

In-vivo release studies:

In this study, the gel formulation used as the control and was prepared by simple dispersion of polymers in water and 10mg of Zidovudine. The animal research study, approved by the animal ethical committee (CPCSEA). Five white rabbits, mean ± weight 2.5kg± 0.05kg, were studied. Before investigations, each rabbit was housed individually in a metal cage with a wire floor. Food and water was supplied ad libitum. During initial catheterization and dosing, each rabbit was placed briefly in rabbit holder and then returned to its own holding cage. Two days before each study, a 10cm x 10cm area on the back of each rabbits was shaved using an electric shaver. One day before each study, a depilatory
washed off, to ensure complete removal of the hairs.

For blood sampling, a catheter was inserted in to the ear artery. After 0.5ml of blood
was withdrawn and discarded, a 1.5 ml sample was collected as the predose control and
placed in a centrifuger with no additives. The catheter was flushed with 2 ml of 0.9% sodium
chloride followed by 0.2 ml heparin solution to avoid the coagulation.

1g of liposomal gel of Carbopol 940 and HPMC K4 100 containing 10mg Zidovudine
were applied to the defined area on the rabbit back. The rabbit was kept in the rabbit holder
during the 24 hours study to prevent it from licking its back and dislodging the catheter from
the ear artery. The blood sampling was repeated, as previously described for the predose
sample, at 0.5, 1 2,3,4,6,8,16,24 hours. After centrifuging for 15 minutes at 3000rpm, plasma
Zidovudine concentrations were analyzed using spectrophotometry at 267nm.

RESULTS AND DISCUSSION:

Liposomes have been used as a carrier system to deliver medications in to the skin in
order to achieve the therapeutic effect with lower systemic absorption. Several novel carrier
systems were suggested to be appropriate for topical drug delivery of liposomes. To achieve
the desirable therapeutic affect of liposomes as drug carriers, they must be loaded with
sufficient amount of active compounds. Therefore liposomes with Zidovudine were prepared
by thin film hydration method with the effects of the formulation variables, lipid phase
composition (Phosphotidyl Choline and cholesterol) and hydration time on the drug
entrainment efficiency, vesicle size and mean distribution of liposomes. Transmission electron
microscope revealed the presence of spherical vesicles (Liposomes). Regardless of the
preparation procedure used, liposomes were of a mean diameter around 1103.509nm in
diameter with the low cholesterol level. The mean particle size and charge of the liposome
were increased with the increased concentration of cholesterol. How ever, liposome prepared
by the thin film hydration method was of a more homogenous size distribution. This was
probably a consequence of the preparation procedure, in the better case.

In this study, liposomes with Zidovudine were mixed in to 2%, 3% Carbopol 940 and
2%, 3% of HPMC and tested for in-vitro, in-vivo release of the entrapped drug by using Franz
diffusion cell for in-vitro release of drug and rabbits were used to study the in-vivo release.

In in-vitro release studies, the Zidovudine conventional liposomes was released 94%
with a period of 8 hours, while gel formulation released 94% of entrapped drug during a
period of 22 hours. As expected, entrapped Zidovudine in a structured vehicle of Carbopol
and HPMC resulted in a prolonged release rate compared to the conventional liposomes due
to the restriction imposed by polymeric network of the gels. The porosity of the gel matrix
allowed intact liposomes and release free drug to diffuse through the matrix in to the receptor
solution (Saline 0.9%w/v). The amount of Zidovudine released from the gel was determined
by spectrometric method. The results show a slower release of Zidovudine from liposomes
incorporated in Carbopol940 and HPMC K4 100 gel.
Table 1: Composition, Hydration time, Mean particle size

<table>
<thead>
<tr>
<th>S. No.</th>
<th>PC: CH (mg)</th>
<th>Drug (mg)</th>
<th>Hydration time</th>
<th>EE</th>
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<tr>
<td>1</td>
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<tr>
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<td>200:100</td>
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<td>5</td>
<td>200:60</td>
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<td>08</td>
<td>51±1.8</td>
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</table>

PC- Phosphotidyl Choline
CH-Cholesterol
EE-Entrapment Efficiency
(n=3)

Table 2: Percentage release of conventional liposomes and Carbopol940 gel 2%, 3%

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Time in hours</th>
<th>% Release from conventional liposomes</th>
<th>% Release from Carbopol 940 2%</th>
<th>% Release from Carbopol 940 3%</th>
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<td>24</td>
<td>98.64</td>
<td>98.47</td>
<td>94.78</td>
</tr>
</tbody>
</table>

(Mean±SD, n=3)
Figure 2: Percentage release of conventional liposomes and Carbopol 940 gel 2%, 3%

Table 2: Percentage release of conventional liposomes and HPMC K4 100 gel 2%, 3%

<table>
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<tr>
<th>S.No.</th>
<th>Time in hours</th>
<th>% Release from conventional liposomes</th>
<th>% Release from Carbopol 940 2%</th>
<th>% Release from Carbopol 940 3%</th>
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<td>23.86</td>
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<td>50.74</td>
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<td>38.1</td>
<td>28.65</td>
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<tr>
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<td>98.64</td>
<td>98.47</td>
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</tbody>
</table>

(Mean±SD, n=3)
Invivo animal studies to be closer to the application of liposomes in humans. An appropriate viscosity of liposomal preparation is required. Carbopol940 2% and HPMC K4 100 2%, showed slower release than Carbopol940 1% and HPMC K4 100 1%, so we took Carbopol940 2% and HPMC K4 100 2% for the animal Invivo studies. The initial release was 38% with period of 8 hours and after it maintained constant around at 44%.

Table-3: Invivo percentage release of HPMC K4 100 2% and Carbopol 940 2%

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Time in hours</th>
<th>Cumulative % Release of HPMC K4 100 2%</th>
<th>Cumulative % Release of Carbopol940 2%</th>
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</thead>
<tbody>
<tr>
<td>1.</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2.</td>
<td>0.5</td>
<td>6.52</td>
<td>5.63</td>
</tr>
<tr>
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<td>8.45</td>
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<td>11.23</td>
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</tr>
<tr>
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</tr>
<tr>
<td>6.</td>
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<td>21.54</td>
<td>16.58</td>
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<td>4</td>
<td>31.87</td>
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<td>30.84</td>
</tr>
<tr>
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<td>10</td>
<td>35.6</td>
<td>31.66</td>
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</table>
Figure-3: In vivo percentage release of HPMC K4 100 2% and Carbopol 940 2%

The slower release is due to the skin permeation of the drug because the drug has to cross several barriers in the skin. The prolong release can be achieved by their incorporation in vehicle suitable for topical self-administration. The prolonged retention on the skin is often required for the desired therapeutic effect; research efforts have been directed to using hydrophilic polymers with adhesive character to improve drug delivery via skin. It has already proven that liposomes are fairly compatible with polymers derived from cross-linked polymers. Therefore the research work confirmed to choose a gel made of Carbopol 940 and HPMC K4 100 polymers as a vehicle for liposomes.

CONCLUSION:

Topical formulations containing Zidovudine-loaded liposomes embedded in to the structured vehicles of Carbopol940, HPMC K4 100 have been prepared and evaluated. The release rates of Zidovudine from the topical gels were affected in the invivo and invitro studies. Comparing the invitro and invivo liposomal gels, the release rate of liposomes entrapped drug was prolonged the release rate of drug. The topical delivery of drug through liposomal gel showed the suitability of delivery systems for prolonged action of topically applied of hydrophilic drugs.

Acknowledgement:

The authors would like to thank Alkem pharmaceuticals, Raigad for providing zidovudine gift sample.
REFERENCES:


SUBJECT: ACCEPTANCE OF PAPER

Dr. S. Palani
Institute of Pharmacy
Bundelkhand University, Jhansi (U.P.)

It is to inform you that your following paper/article has been peer reviewed by the expert referees and accepted for publication as full paper/short communication in Indian Journal of Applied Life Sciences Vol.3 (No. 1)

Effects of pH, Salt, Temperature on conventional liposomes size enlargement analyzed by optical microscope

Yours Sincerely

[Signature]

Dr. Minik Sharma
(Editor)
Effects of pH, Salt, Temperature on conventional liposomes size enlargement analyzed by optical microscope

P.K.SHRAMA, S.PALANI*, R. IRCHHIAYA
Institute of Pharmacy, Bundelkhand University, Jhansi-284003

ABSTRACT:

The objective of the present study was to investigate the effects of experimental conditions like pH, salt, temperature on the interactions of conventional liposomes. The model drug Zidovudine was entrapped in the liposomes composed of Phosphatidyl Choline and cholesterol in the ratio of 200mg: 80mg and prepared by thin film hydration method. Liposomes preparation was characterized and compared for particle size, mean dispersion, entrapment efficiency, DSC, Zeta potential and the size enlargement studies were carried out in optical microscope by using Magnus.

Key words: Liposomes, zidovudine, temperature, size enlargement, pH, salt.

INTRODUCTION:

Liposomes are becoming increasingly important as carriers of biologically important molecules in living system. (Tyrell et al., 1976; Papahadjopoulos, 1978; Gregoriadis, 1980; Roerdink et al., 1981). Liposomes have a variety of routes of administrations including oral, dermal, intravenous, ophthalmic (Labris et al, 2003). Liposomes mimic cells and are used for encapsulation and sustained release of drugs in modern therapies. They are aggregates containing a continuous bilayer of phospholipids around in aqueous space. There has been much interest in liposomes as drug delivery carriers, not only because of wide range of biologically active substances can be encapsulated, but that can be administered to man or animals with out any adverse effect. Incorporation in to liposomes can reduce the toxicity and produce sustained release (Genin et al). Liposomes, after three decades of research, are still gaining increasing interest with special emphasis more recently on their use as drug carrier systems. For therapeutic purpose they must be loaded with active substances. This is more achieved with lipophilic and hydrophilic molecules as they have tendency to be incorporated in the liposomal membrane. This is more essential achieved with amphiphile molecules like phospholipids and cholesterol as they have a tendency to be incorporated in the liposomal membrane. In contrast, hydrophilic molecules must be encapsulated in the aqueous interior, which in general, cannot easily be performed in an efficient manner (M.J.Ostro1987). A variety of liposomes preparations were introduced and they have to fulfill the basic requirements (a) Produced liposomes should be homogeneous (b) Efficient of
encapsulation of hydrophilic drugs. Where as thin film hydration technique achieve high efficiency and easy method to produce in the laboratories (R.L. Juliano et al). The concept of this project was fairly to entrap hydrophilic drugs like zidovudine. The ratio of volume inside the liposomes compared to the total aqueous volume of the preparation is encapsulation efficiency. The increasing lipid concentration more liposomes per unit volume of the preparation are formed. When phospholipid dispersed in aqueous medium at certain concentrations results in highly viscous dispersions up to semisolid consistency. So, the phospholipid, cholesterol and hydration times were changed to achieve maximum entrapment efficiency. The prepared liposomes were investigated at different temperature, pH and salt concentration for their stability.

Zidovudine, the first anti HIV compound approved for clinical use is widely used for the treatment of AIDS either alone or combination with other antiviral agents. However, the main limitation to therapeutic effectiveness of zidovudine its dose dependant hematological toxicity, low therapeutic index, short biological half life and poor bioavailability (K.D.Kieburutz et al). Zidovudine available in the market as conventional tablet form. After oral administration, it is rapidly absorbed from GIT exhibiting a peak plasma concentration of 1.2μg/ml at0.8 hours (R.W.Klecker et al). In the systemic circulation, it is first converted to Azidothimidine triphosphate, which is pharmacologically active and prevents the replication of the HIV virus. The biological half-life of zidovudine is 4 hours.

MATERIALS AND METHODS:

Materials:

The materials used in the study were Zidovudine a gifted sample of Alkem Laboratories Ltd, Raigad. Phosphotidyl choline, Qualigens, Mumbai. Cholesterol, Qualigens, Mumbai. Chloroform, Merck, Mumbai. Methanols, Qualigens, Mumbai were Purchased. All in the study, other ingredients used were of analytical grade.

Methods:

Preparation of liposomes:

Multilammellar liposomes were prepared by using thin film hydration method (A.D.Bangham et al)) Accurately weighed quantity of Drug, Phosphotidyl Choline, Cholesterol was taken. Phosphotidyl Choline, Cholesterol in the ratio of 200mg: 80mg were dissolved in two parts of chloroform and one part of methanol mixture in 250 ml spear shaped flask (G.Gregoriadis et al). The chloroform and methanol mixture was evaporated at 45°C under reduced pressure (600psi) at 150rpm using Rota evaporator. After the chloroform and methanol was completely evaporated. The flask was kept overnight under vacuum to remove residual solvent. The thin film was hydrated using drug-containing 0.9%w/v Saline solution for 8 hours until vesiculation was completed.
Estimation of Entrapped Drug in Liposomes

Zidovudine entrapped within the liposomes was estimated after removing the unentrapped drug. The unentrapped drug was separated from the liposomes by subjecting the dispersion to configuration in a cooling centrifuge (Remi Equipments, Mumbai, India) at 15 000 rpm at a temperature of −4°C for 30 minutes, whereupon the pellets of liposomes and the supernatant containing free drug were obtained (Kulkarni et al). The liposome pellets were washed again with distilled water to remove any unentrapped drug by centrifugation. The supernatant was analyzed for the drug content after suitable dilution with saline solution by measuring absorbance at 267 nm using Shimadzu U-V 1700(U-V Spectrophotometer). The percentage drug entrapment in the liposomes was calculated from the difference between the initial drug added and the drug detected in the supernatant.

Transmission electron microscopy:

Zidovudine liposomal dispersion was examined by Transmission electron microscope to find out the presence of spherical vesicles and type of vesicles.

Determination of zeta potential:

The zeta potential is the overall charge a particle acquires in a particular medium. Both mean particle size and charge of the particles can be measured on a Malvern Zetasizer. The zeta potential of a liposome preparation can help to predict the fate of the liposomes in vivo and invitro. Any subsequent modification of the liposome surface can also be monitored by measurement of the zeta potential (D.J.Crommelin et al).

RESULTS AND DISCUSSION:

Liposomes have been used as a carrier system to deliver medications in to the skin in order to achieve the therapeutic effect with lower systemic absorption. Several novel carrier systems were suggested to be appropriate for topical drug delivery of liposomes. To achieve the desirable therapeutic affect of liposomes as drug carriers, they must be loaded with sufficient amount of active compounds. Therefore liposomes with Zidovudine were prepared by thin film hydration method with the effects of the formulation variables, lipid phase composition (Phosphotidyl Choline and cholesterol) and hydration time on the drug entrapment efficiency, vesicle size and mean distribution of liposomes. Transmission electron microscope revealed the presence of spherical vesicles (Liposomes). Regardless of the preparation procedure used, liposomes were of a mean diameter around 1110.509nm in diameter with the low cholesterol level. In the optical microscope it was found that the average diameter of the liposome vesicle was 2.467µm. The mean particle size and charge of the liposome were increased with the increased concentration of cholesterol. How ever, liposome prepared by the thin film hydration method was of a more homogenous size distribution. This was probably a consequence of the preparation procedure, in the better case.
Table-1: Composition, Hydration time, Mean particle size

<table>
<thead>
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<th>S. No</th>
<th>PC: CH (mg)</th>
<th>Drug (mg)</th>
<th>Hydration time</th>
<th>EE</th>
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<td>51±1.8</td>
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</tbody>
</table>

PC- Phosphotidyl Choline
CH-Cholesterol
EE-Entrapment Efficiency (n=3).

Effect of temperature of liposomes size enlargement:

The conventional liposomes containing zidovudine were taken and stored at different temperatures like 4°C, 25°C, 37°C, and 50°C for 30 days. The influences of temperature on liposomes diameter as a function of time have been investigated and are shown in the fig-1 and values of the size enlargement of liposomes shown in table-2. In the higher temperatures, the liposomes may aggregates and form clusters. This investigation is to find out the effects on size enlargement of liposomes and its suitable storage temperature. The liposomal coalescence is not reversible and it is sensitive to temperature(B.L.Gamon et al).

The aggregation of liposomes due to Brownian motion while increasing the temperature, there will be increasing in motion of the liposomes vesicles. The reaction limited clusters aggregation process, the average cluster size grows exponentially with the time. After aggregation liposomes tends to form large vesicles via coalescence. In this case that the decreasing the temperature of zidovudine liposomes increased the viscosity of liposomal suspension and the liposomal coalescence rate decreased. So it is shown in this study that a low temperature i.e. 4°C increases the stability of liposomes in relation to their size.

Table-2 Effect of temperature on liposome size enlargement

<table>
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<th>S.No</th>
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Effect of pH on liposomes size enlargement:

The conventional liposomes containing zidovudine were stored at different values like 3, 5, 7, 8. The influences of pH on liposomes diameter as a function of time have been investigated and are shown in fig-2 and values of the size enlargement of liposomes shown in table-3. It was found that the effect of pH on liposome stability is significant (D.D. Lasic et al). The pH range above 5-8, there is no significant change in the size enlargement. But the decreased pH (pH-3), the rate of membrane fusion may occur in the neutral liposomes.

Table-3: Effect of different pH on liposomes size enlargement

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Effect of calcium chloride on liposomes size enlargement:

The conventional liposomes containing zidovudine were stored at different molar ratio of calcium chloride concentration, i.e. 0.001M, 0.01M, 0.1M solution at 25°C at pH7 reported (M. Juan Ruso et al). The influences of pH on liposomes diameter as a function of time have been investigated and are shown in fig-2 and values of the size enlargement of liposomes shown in table-4. in the range of 0.001M to 0.01M there is no significant change in the size enlargement. Above 0.01M i.e. 0.1M calcium chloride salt may induce aggregation behavior of conventional liposomes occurs only at a high concentration salt level.

Table-4 Effect of 0.001M, 0.01M, 0.1M CaCl₂ on liposomes size enlargement

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<th>S.No</th>
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**Table 4**: Effect of 0.001M, 0.01M, 0.1M CaCl$_2$ on liposomes size enlargement

![Graph showing effect of different CaCl$_2$ concentrations on liposomes size enlargement over time.]

**CONCLUSION**

The stability of liposomes with respect to aggregation was evaluated by using different salt concentrations, different pH and different temperatures. With the increasing concentration of the electrolytes were more effective for liposome aggregation. The highest level of stability of liposomes was observed at pH-7. The temperature dramatically influenced the aggregation. Low temperature was suitable for the storage of liposomes.

**ACKNOWLEDGEMENTS**

The authors would like to thank Alkem pharmaceuticals, Raigad for providing zidovudine gift sample.

**REFERENCES:**


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Chief Editor
ABSTRACT

The evolution of the science and technology of liposomes as a drug carrier has passed through a number of distinct phases. Because they exhibit peculiar properties due to their structure, chemical composition amphiphile nature, physico-chemical characters and colloidal size, which are used in various applications. These properties point to several applications as the solubilizer for insoluble drugs, dispersants, and sustained release system, delivery system for the encapsulated substance, stabilizer, protective agents, and micro reactive being the most obvious ones. Yet interest in liposomes, especially among academic workers, spread rapidly we attribute this to the remarkable structural versatility of the system, which enables the design of countless liposomes versions to satisfy particular needs in terms of both technology and optimal function in vivo.

INTRODUCTION

Liposomal vesicles were prepared in the early years of their history from various lipid classes identical to those present in most biological membranes (Abraham W. et al.). Liposomes were discovered in the mid of 1960's and originally studied as cell membrane model Paul Ehrlich coined the term "magic bullet" in 20th century where carrier system's was proposed to simply carry the drug to its of action and releasing it selectively while non target sites should absolutely be exempted from drug effect (Crommelin O.J.A. et al.). The exploration and progressive advent of liposomal drug delivery system has rekindled interest in magic bullet approach, for surely man's ingenuity can find means for directing these drugs filled packed or lipid bilayer vesicles to specific cell or anatomical sites within the body (Barenholz Y. et al.). Liposomes were described as a model of cellular membranes and quickly were applied to the delivery of substances to cells. Liposomes are microscopic spherical vesicles that form when phospholipids are hydrated. When mixed in water under low shear conditions, the phospholipids arrange themselves in sheets, the molecules aligning side by side in like orientation, "heads" up and "tails" down. These sheets then join tails-totails to form a bilayer membrane, which encloses some of the water in a phospholipids sphere (Suggy S. et al.).
Mechanism of liposomal formation

Liposomes are formed open hydration of lipid molecules normally lipids are hydrated from a dry state (thin or thick lipid film, spray dried powder), and stacks of crystalline bilayers become fluid and swell myelin-long, thin cylinders grow and upon agitation detach set close in to large, multilamellar liposomes because this eliminates unfavorable interactions at the edges. Once the large particles are formed they can be either broken by mechanical treatment in to smaller bilayered fragments, which close into smaller liposomes (Lasic D. D. et al.).

Classification of liposomes

Multilamellar vesicles (MLV) consist of several (up to 14) lipid layers (in an onion-like arrangement) separated from one another by a layer of aqueous solution. These vesicles are over several hundred nanometers in diameter. Small unilamellar vesicles (SUV) are surrounded by a single lipid layer and are 25-50 nm (according to some authors up to 100 nm) in diameter. Large unilamellar vesicles (LUV) are, in fact, a very heterogeneous group of vesicles that, like the SUVs, are surrounded by a single lipid layer. The diameter of these liposomes is very broad, from 100 nm up to cell size (giant vesicles) (Baiydia et al) Besides the technique used for their formation the lipid composition of liposomes is also, in most cases, very important (Allen T. M. et al.; Swarbrick J. et al.).

Methods of liposomes preparation

Basic studies on liposomal vesicles resulted in numerous methods of their preparation and characterization (Storm G. et al.; Vyas S P. et al.).

A. Hydration stage

1. Mechanical methods
   
   • Vortexing or hand shaking of phospholipid dispersions (MLV)
   • 'Microfluidizer' technique (mainly SUV)
   • High-shear homogenization (mainly SUV)

2. Methods based on replacement of organic solvent(s) by aqueous media
   
   • Removal of organic solvent(s) before hydration (MLV, OLV, SUV)
   • Reverse-phase evaporation (LUV, OLV, MLV)
   • Use of water immiscible solvents: ether and petroleum-ether infusion (solvent vaporization) (MLV, OLV, LUV)
   • Use of water miscible solvents such as ethanol injection (MLV, OLV, SUV)

3. Methods based on detergent removal
   
   • Gel exclusion chromatography (SUV)
   • 'Slow' dialysis (LUV, OLV, MLV)
   • Fast dilution (LUV, OLV)

4. Methods based on size transformation and fusion
   
   • Spontaneous fusion of SUV in the gel phase (LUV)
B. Sizing stage
   1. High pressure extrusion
   2. Low pressure extrusion
   3. Ultrasonic treatment

C. Removal of non-encapsulated material
   1. Dialysis
   2. Ultracentrifugation
   3. Gel-permeation chromatography
   4. Ion-exchange resins

Targeting via Liposomes

Depending on the need, one can use SUV type or MLV type vesicles for effective entrapment and delivery of the drug to the target tissues or cells (Baidya S. et al.). Nevertheless, charge properties and interactions of the active compound with vesicle forming molecules will determine the effect of entrapment, i.e., the amount of the compound that can be "loaded" into a single vesicle (Gabizon A. et al.). On the other hand, the composition of the molecules used for the formation of the vesicular structure will, at least, affect the fate of vesicles from the site of their introduction as well as the interaction with component of the body (e.g., surface charge, serum proteins, lipoproteins, opsonin system, phagocytic system and finally target cells. In the earlier studies, when therapeutically active substances were not easily available, most of the experiments were done using a marker compound (Bandak S. et al.). The results, however, were not the same as those obtained in experiments in which an active substance was used and the conditions were more related to the real situation (ex-vivo, in-vivo). These findings implicate the necessity for studies in which an active substance is used and the conditions of the experiments resemble, as closely as possible, those of therapeutic Liposomal (vesicular) drug application. The benefits of liposomal formulations were already demonstrated clinically and stimulate many laboratories (research and pharmaceutical) in their efforts to introduce new Liposomal vesicular drugs (Lasic DD. et al.).

Liposomes are well established as drug carriers in topical treatment of diseases, especially in dermatology. They can enhance penetration of encapsulated hydrophilic drugs into the skin to enable a proper therapeutic effect. Because of they are able to carry with them any enclosed substances into the dermis and to ‘the individual cells (Zhang YP et al.).

Sustained release of the incorporated drug,

Liposomes are typical vehicles, which are able to transport dermatological and cosmetic active agents of different types. The active agents are encapsulated and protected against environmental influences. Liposomes spread out excellently in the horny layer of the skin and form depots of active agents. Aqueous dispersions show a clear to milky appearance
according to the size of the Liposomes. Similar to the horny layer of human skin, liposomes consist of one or several bilayers of phosphatidylcholine. Liposomes without active agents ("empty liposomes") show all dermatological and cosmetic effects of phosphatidylcholine (Zuidam NJ. et al.; Vernooij EAAM et al.).

The size of these spheres is very small, in the order of a nanometer. As illustrated, the spheres are hollow inside and enclose some of the liquid material in which they were formed (inclusion). Because of the small size of the phospholipid molecule and microspheres, they can pass through the epidermis and act as a carrier for the enclosed substances.

**Release Kinetics of Liposomal Payload**

Liposomes are most useful for being able to transfer and deliver active ingredients to the application site of formulation. The liposome wall is very similar, physiologically, to the material of cell membranes. Application of formulation over skin area causes deposition of liposomes on the skin and begins to merge with the cellular membranes. In the process, the liposomes release their payload of active materials into the cells. As a consequence, not only is delivery of the actives very specific directly into the intended cells but also the delivery takes place over a longer period of time. Liposomes exhibit better stability, penetration and efficacy at lower usage levels (Lasic DD. et al.).

Liposomes as a delivery system can be made to release their payload under a variety of conditions.

- Slow / Fast Release of Hydrophilic Payload
- Slow / Fast Release of Hydrophobic Payload
- Bilayer Composition
  - Chain Length
  - Saturation
  - Lipid Class
- Physical Configuration of Liposome
- Solvent-Dependent Release
- pH-Dependent Release
- Temperature-Dependent Release

**Advantages of Liposomes**

The characteristics of liposomes also yield a variety of other formulation benefits (Daan J. A. et al.).

- Controlled Delivery System
- Biodegradable, Non-Toxic
- Carry Both Water and Oil Soluble Payloads
Applicability of Liposomes

One may conclude that, at present, the term "liposomes" covers not only phospholipid based vesicles but also other vesicular structures with properties identical or similar to those of classical, natural phospholipid based Liposomes. In the early 70's the use of liposomes as a drug carrier system was proposed by Gregoriadis & Ryman. Since this first report, liposomes were developed as an advanced drug delivery vehicle. They are generally considered non-toxic, biodegradable and non-immunogenic (Osborne D. W. et al.). Associating a drug with liposomes markedly changes its pharmacokinetics and lowers systemic toxicity; furthermore, the drug is prevented from early degradation and/or inactivation after introduction to the target organism (Gabizon A. et al.). The use of liposomes or, in general, vesicular structures for the delivery of various active compounds is recognized in relation to water solubility of the compound. When the compound is water soluble, the size and volume of the aqueous compartment of the vesicle is crucial (Daan J. A. et al.). In contrast, hydrophobic compounds will prefer incorporation into the lipid (amphiphile) layer that constructs the vesicle. In such a case, the size of the aqueous compartment is not important.

Therapeutic applications of liposomes

Liposomes are used for the following range of therapeutic and pharmaceutical applications (Vyas S P. et al.):

1. Liposomes as drug protein delivery vehicles.
   - Controlled and sustained drug release in situ.
   - Enhanced drug solubilization.
   - Altered pharmacokinetics and biodistribution.
   - Enzyme replacement therapy and lysosomal storage disorders

2. Liposomes in antimicrobial and antifungal (lung therapeutics) and antiviral (anti-HIV) therapy
   - Liposomal drugs
   - Liposomal biological response modifiers

3. Liposomes in tumour therapy
   - Carrier of small cytotoxic molecules
   - Vehicle for macromolecules as cytokines or genes
4. Liposomes in gene delivery.
   - Gene and antisense therapy
   - Genetic (DNA) vaccination

5. Liposome Immunology.
   - Immunoadjuvant
   - Immunomodulator
   - Immunodiagnosis

6. Liposomes as artificial blood surrogates.

7. Liposomes as Radiopharmaceutical and Radiodiagnostic carriers

8. Liposomes in cosmetics and dermatology

9. Liposomes in enzyme immobilization and bioreactor technology.

CONCLUSION

Liposomes have been realized as extremely useful carrier systems, additive(s) and tools in various scientific domains. Thus, liposomes over the years have been investigated as the major drug delivery systems due to their flexibility to be tailored for varied desirable purposes. The flexibility in their behavior can be exploited for the drug delivery through any route of administration and for-any drug or material irrespective of its physicochemical properties (Swarbrick J. et al.). The uses of liposomes in the delivery of drugs and genes to tumour sites are promising and may serve as a handle for focus of future research (Storm G. et al.).

REFERENCES

- Barenholz Y, Crol11mclin DJA. Liposomes as pharmaceutical dosage forms.

• Daan J. A. Crommelin and Gert Storm, 2003, Liposomes: From the Bench to the Bed; Journal of Liposome research Vol. 13, No. 1, pp. 33-36,


• Lasic D D., July 1998; Novel application of liposomes, Tibtech, Vol.16.


• Osborne D. W., Anton H. Anann; Topical Drug Delivery formulations; Vol. 42, Marcel Dekker Inc.


• Suggy S. Murari C.R., and Ahmad I., Liposomes (a review). Nov.2004 Biopharm.


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