Proniosomes as a Drug Carrier for Transdermal Delivery of Herbal Drug

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ABSTRACT

Over many millennia, natural products were the only means for food, to treat diseases and injuries. Recently in the field of pharmaceutical sciences great efforts are being directed towards herbal drugs by incorporating them in already existed drug delivery system to solve the problem related to poor solubility, poor bioavailability, dosing problem, stability, toxicity etc. Curcumin is a yellow pigment isolated from the rhizome of perennial herb Curcuma longa family Zingiberaceae and has been popularly used as a food additive. Transdermal drug delivery has been recognized as an alternative route to oral delivery. Proniosomes offer a versatile vesicle delivery concept with the potential for drug delivery via the transdermal route In present study proniosomal carrier system of curcumin for transdermal delivery were developed by encapsulating curcumin in Tween 60, cholesterol, diethyl ether by ether injection method. The formulated systems were characterized for size, vesicle count, drug entrapment, angle of repose, rate of hydration, drug release profiles and vesicular stability at different storage conditions. In-vitro release studies were performed by using albino rat skin for 24 hrs.

Key words: Herbal Drugs, Proniosomes, Curcumin, In-vitro release, TDDS, Stability studies.

INTRODUCTION

Non ionic surfactant vesicles known as niosomes are gaining great attention as an alternative potential drugs delivery system to conventional liposomes. Niosomes have shown advantages as drug carriers, such as being cheap and chemically stable alternative to liposomes, but they are associated with problems related to physical stability, such as fusion, aggregation, sedimentation and leakage on storage[1-3]. The pronosome approach minimizes these problems by using dry, free flowing product, which is more stable during sterilization and storage[3]. The plant Curcuma longa (Zingiberaceae) commonly called as Indian saffron. Turmeric in English, Haridra in Sanskrit, Haldi in hindi, paspu in telugu, manjal in Tamail, Halada in Guajarati, Halade in Marathi, Mannal in Malayalam and Arishina in Kannada[4].

Figure: 1. Structure of Curcumin

Curcumin [(1E, 6E)-1, 7-bis (4-hydroxy-3-methoxyphenyl) -1, 6-heptadiene-3, 5-Dione] is the active ingredient of the spice turmeric, used in cooking in India and other regions of Asia. It has a long history as an herbal remedy for a variety of diseases and was used in Indian and Chinese traditional medicine as early as 700 AD[6]. Curcuminoids, the oleoresins, derived from ethanolic extraction of turmeric are mainly responsible for yellow color and are considered responsible for the biological activity. A vast research revealed curcumin has a wide spectrum of therapeutic effects such as anti-inflammatory, antibacterial[7], antifungal[8], anticancer[9] antispasmodic[10], antioxidant[11], antiamoebic[12], anti HIV[13], antidiabetic[14], and antifertility[15] etc. It is also reported that the curcumin is safe up to 8g/day. Curcumin was selected for the studies as it is poorly absorbed in the lower GIT and has short elimination half-life (~0.39 h). The poor bioavailability (< 1%) of the molecule owing to the insolubility at gastric pH and degradation at alkaline pH of intestine in the human body has severely limited its clinical application. High oral doses (8 g/day) in humans result in Cmax of < 2 μM, and short half life (~28 minutes) limits its use by oral route[16]. Several studies of curcumin indicate its poor solubility, very low GIT dissolution rate, low absorption, and its extensive systemic metabolism are the reasons for its delivery problems and lack of clinical success[17]. Thereby, the present study aims at designing a new transdermal formulation for curcumin characterized by safety and high therapeutic efficacy, through designing an optimum proniosome gel formulation so as to reduce the daily administered dose of Curcumin with a subsequent improvement in patient compliance and drug safety.

MATERIALS AND METHODS

Curcumin was procured as the gift sample from Krish Enterprises, Mumbai, India. Span 80 was purchased from CDH, Delhi. All other chemicals used were of analytical grade.

Ether Injection Process

Proniosomes containing curcumin of 1:1 ratio was prepared by taking cholesterol, Tween 60 in a 50ml beaker. The mixture was dissolved in diethyl ether and the solution was slowly injected into a beaker containing curcumin in phosphate buffer saline (pH 7.4). The temperature maintained during the injection was 40-60°C. The differences in temperature between phases cause rapid vaporization of ether resulting in spontaneous vesiculation[18].

Table1. Composition of formulations

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Formulation Code</th>
<th>Diethyl ether (ml)</th>
<th>Drug (mg)</th>
<th>Surfactant: Cholesterol ratio</th>
<th>Tween 60 (mg)</th>
<th>Cholesterol (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PC1</td>
<td>2</td>
<td>100</td>
<td>1:4</td>
<td>50</td>
<td>200</td>
</tr>
<tr>
<td>2</td>
<td>PC2</td>
<td>2</td>
<td>100</td>
<td>1:3</td>
<td>50</td>
<td>150</td>
</tr>
<tr>
<td>3</td>
<td>PC3</td>
<td>2</td>
<td>100</td>
<td>1:2</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>PC4</td>
<td>2</td>
<td>100</td>
<td>1:1</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

Evaluation

Vesicle Size Determination

It was carried out using an optical microscopy with a calibrated eyepiece micrometer. About 200 niosomes were measured individually, average was taken, and their size range, mean diameter were calculated[19].

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Drug Content
Prioniosomes preparation equivalent to 40 mg of curcumin was taken into a standard volumetric flask. Then they were lyzed with 100ml of phosphate-1-ol by shaking. Then 1ml of this was subsequently diluted with phosphate buffer (pH 7.4). The absorbance was measured at 254 nm and calculated drug content from the calibration curve of curcumin in phosphate buffer Ph 7.4[20].

Scanning Electron Microscopy (SEM) studies
Pure drug and selected formulation were sputtered coated using pelco gold coated SEM stubs. The surface morphology of the layered sample was examined using SEM. The sample was placed in an evacuated chamber and scanned in a controlled pattern by an electron beam. Interaction of the electron beam with the specimen produces a variety of physical phenomenon that detected, are used to form images and provide information about the specimens[20].

Drug Entrapment efficiency of niosomes
Entrapment efficiency of niosomes was determined by exhaustive dialysis method. The measured quantity of niosomal suspension was taken into a dialysis tube to which osmosis cellulose membrane was securely attached on one side. The dialysis tube was suspended in 100ml phosphate buffer (pH 7.4), which was stirred on a magnetic stirrer. The unentrapped drug was separated from the niosomal suspension into the medium through osmosis cellulose membrane. At every hour entire medium (100ml) was replaced with fresh medium (for about 9-12hrs) till the absorbance reached a constant reading indicating no drug is available in unentrapped form. The niosomal suspension in the dialysis tube was further lyzed with propane-1-ol and estimated the entrapped drug by UV spectrophotometric method at 254 nm. The entrapment efficiency was calculated using following equation[21]

\[
\text{% Entrapment} = \frac{\text{Total drug} - \text{Diffused drug}}{\text{Total drug}} \times 100
\]

Measurement of Angle of repose
Angle of repose of dry proniosome of curcumin powder was measured by a funnel method. Proniosomes powder was poured into a funnel which was fixed at a position so that the 12mm outlet orifice of the funnel is 5cm above a level black surface. The powder flows down from the funnel to form a cone on the surface. Angle of repose was then calculated by measuring the height of the cone and the diameter of its base[21].

Rate of hydration (Spontaneity)
Spontaneity of niosomes formation is described as number of niosomes formed after hydration of proniosomes for 15 min. Proniosomes were transferred to the bottom of a small stoppered glass tube and spread uniformly. One ml saline (0.154 M NaCl) was added carefully along the walls of the test tube and kept aside without agitation. After 15-20 min a drop of aqueous layer was withdrawn and placed on Neubauer’s chamber. The number of niosomes eluted from proniosomes was counted[21].

Stability Studies:
The ability of vesicles to retain the drug (drug retention behaviors) was assessed by keeping the proniosomal gel at three different temperature conditions, i.e., refrigeration temperature (4-8°C), Room Temperature (25±2°C) and Oven (45±2°C). Throughout the study, proniosomal formulations were stored in aluminium foil-sealed glass vials. The samples were withdrawn at different time intervals over a period of one month and drug leakage from the formulations was analyzed for drug content spectrophotometrically[22].

In-vitro skin permeation study
The in vitro rat skin permeation study was carried out as per the guidelines compiled by CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animal, Ministry of Culture, Government of India) and all the study protocols were approved by the local institutional Animal Ethics Committee (PSIT, Kanpur, India). Also an international protocol for conducting experiments on animals was followed[21]. The abdominal hair of albino rats (wistar strain), weighing 200 ± 20 gm, was shaved using hand razor. Care was taken not to damage the skin surface. Rat was sacrificed by administration of excess chloroform inhalation and the abdominal skin of the rat was separated. The skin was stored at -20°C and used within three days for the permeation study. It has been reported that storage in the refrigerator keeps the metabolic activity of the skin. Before the permeation study, the skin was hydrated in phosphate buffer pH 7.4 (containing 0.02% sodium azide as a preservative) at 4°C over night and the adipose tissue layer of the skin was removed by rubbing with a cotton swab. The permeation of drug from proniosomal gel formulations was determined by using Franz diffusion cell. The excised rat skin was mounted on the receptor compartment with the stratum corneum side facing up-wards into the donor compartment. The donor compartment was filled with the proniosomal gel formulation. A 15 ml of pH 7.4-phosphate buffer containing 10% PEG was used as receptor medium to maintain a sink condition. The available diffusion area of cell was 3.14 cm². The receptor compartment was maintained at 37 ± 1°C, with magnetic stirring at 600 rpm. The samples from the receptor compartment were withdrawn at predetermined time intervals and immediately replaced by an equal volume of fresh buffer solution. Initial experiments confirmed the maintenance of sink condition by this procedure. The samples withdrawn from the receptor compartment were then analyzed by using UV spectrophotometer.

Statistical Analysis:
To ascertain the drug release mechanism and release rate, data of the above formulations were model fitted using PCP Disso V3.0 dissolution software. The models selected were Zero order, Higuchi Matrix, Korsemayer Peppas. The regression coefficient values for all these models are shown in Table 3. In all the cases the best fit model was found to be Peppas with ‘n’ value between 0.65 to 0.73 suggesting the non fickian (anomalous) release mechanism for the drug i.e., erosion followed by diffusion controlled. The study of drug release kinetics showed that majority of the formulations governed by Peppas model. The curve was obtained after plotting the cumulative amount of drug released from each formulation against time[24].

RESULTS
Prepared niosomes reveal that they are discrete and spherical shape, and some vesicles are slightly elongated. The prioniosomes prepared were in the size range of 3.28-4.54 µm. The entrapment efficiency was 84.3% for formulation PC4 whereas it was 76.2%, 78.4%, and 80.2% for formulations PC2, PC3, PC4, respectively. Angle of repose of the formulation was lies in between 33.32° and 39.21° (table 2). Maximum drug content was present in formulation PC1 containing 86%. Rate of spontaneous lies in between 12.16 and 14.20. Stability studies performed on selected formulations PC2 shows 99.59% drug content at refrigeration condition, 93.73 % drug content at oven condition and 98.18% drug content at room temperature for the studies performed for 4 week on the formulations (figure 3).

In-vitro skin permeation study performed on albino rat skin shows maximum release for formulation PC4, 85.034 %, and 74.476%, 63.649%, 54.323 % for PC1, PC2 and PC3 respectively during the studies performed for 4 week on the formulations (figure 3).
The results showed that cholesterol forms a film around the vesicles and increases the micro viscosity of the bi-layer. The thickness of the film depends upon the concentration of Cholesterol. This shows inclusion of cholesterol improves drug retention time and thus reduces permeability.

CONCLUSION
It is evident from this study that proniosomes are a promising prolonged delivery system for curcumin and have reasonably good stability characteristics. In the recent days, herbal medicines have got popularity all over the world. Incorporation of herbal drugs in novel drug delivery system may lead to an excellent result. In this investigation, proniosomal systems of curcumin and surfactant tween 60 which are easily available and cheap are easily prepared. The formulation procedure is simple and does not involve lengthy procedures and use of several pharmaceutically excipients. Through the present experimentation, it has found that the drugs of herbal origin can be utilized in a better form with enhanced efficacy by incorporating in modern dosage forms. This experimentation is one of the first few attempts to utilize ayurvedic drugs through TDDS. Use of turmeric in TDDS can be also considered as a new version of ayurvedic turmeric poultice or lepa. In conclusion, we can state that besides imparting controlled systemic transdermal delivery to curcumin, proniosomal gel possesses high entrapment efficiency.

REFERENCES

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