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Research Article

Development and Evaluation of Proniosome-Encapsulated Curcumin for Transdermal Administration

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Abstract

Purpose*: To develop a proniosomal carrier system of curcumin for transdermal delivery.*
Methods*: Proniosomes of curcumin were prepared by encapsulation of the drug in a mixture of Span 80, cholesterol and diethyl ether by ether injection method, and then investigated as a transdermal drug delivery system (TDDS). The formulated systems were characterized for size, drug entrapment, angle of repose, hydration rate and vesicular stability under various storage conditions. In vitro release studies were performed using albino rat skin.*
Results*: The method used for preparing proniosome resulted in an encapsulation yield of 82.3 – 86.8%. Scanning electron microscopy analysis showed that the surface of the particles was smooth. Stability data following storage under different conditions showed that the drug content of the proniosomes varied from 99.5% under refrigerated condition to 99.2 and 93% at room and elevated temperatures, respectively. One of the formulations (PG1) showed prolonged in vitro drug release of 61.8% over a period of 24 h.*
Conclusion*: It is evident from this study that proniosomes are very stable and promising prolonged delivery system for curcumin.*

Keywords: *Proniosomes, Curcumin, Drug stability, In vitro release, Transdermal drug delivery.*

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INTRODUCTION

Niosomes or nonionic surfactant vesicles are microscopic lamellar structures formed on admixtures of nonionic surfactant of the alkyl or dialkyl polyglycerol ether class and cholesterol with subsequent hydration in aqueous media [1]. Since the structure of the niosome accommodate hydrophilic, lipophilic as well as ampiphilic drug moieties, they can be used as delivery device for various drugs [2]. Proniosome gel is an intermediate state of formation of niosome. The niosomes can be prepared from the proniosomes by adding the aqueous phase with the drug to the proniosomes with brief agitation at a temperature above the mean transition temperature of the surfactant [3]. Proniosome possess different advantages over niosome i.e. it is not susceptible to instability problems like aggregation, fusion, leaking and hydrolysis of encapsulated drugs which limits the shelf life of the dispersion[4].

Curcuminoids are oleoresins, derived from the ethanolic extraction of turmeric. They are primarily responsible for the yellow coloration as well as biological activity. Earlier studies have shown that curcumin has a wide range of therapeutic effects such as anti-inflammatory [5] , antibacterial [6], antifungal [7], anticancer [8] antispasmodic [9], antioxidant [10], antiamoebic [11] , anti HIV [12], antidiabetic [13] , antifertility [14] etc. It is reported to be very safe with a toxicity profile of 8 g/day. Studies on the pharmacokinetics of curcumin indicates its poor solubility in acid medium and consequently very low absorption from the GIT. Furthermore, there is extensive systemic metabolism hence its delivery problems and lack of clinical success [15]. This ultimately leads to poor bioavailability by oral administration. Curcumin is already in use by topical and oral administration. By the means of transdermal drug delivery system all these can be avoided and therapeutic efficacy of curcumin can be improved. In this present study, proniosomal gel of curcumin have been prepared for transdermal drug delivery.

EXPRIMENTAL

Materials

Curcumin was obtained as the gift from Krish Enterprise, 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 (PG4) were prepared by dissolving cholesterol (50 mg) and Span 80 (50 mg) in 2 ml diethyl ether and the solution was injected slowly (0.25 ml/min) into a beaker containing 200 mg curcumin in 10 ml phosphate buffer (pH 7.4). The temperature was maintained at 40 – 60 ^{0}C during the injection. The differences in temperature between the phases caused rapid vaporization of the ether resulting in spontaneous vesiculation [16,17]. Three other formulations were similarly prepared using cholesterol and Span 80 in the ratios 1:2, 1:3 and 1:4 (see Table 1)

Table 1: Composition of formulations*

Formulation Code	Span 80 (mg)	Cholesterol (mg)	Span/ Cholesterol ratio
PG1	50	200	1:4
PG2	50	150	1:3
PG3	50	100	1:2
PG4	50	50	1:1

Each formulation contained diethyl ether (2 ml), drug (50 mg)

Vesicle size determination

An optical microscope (model) with a calibrated eyepiece micrometer was used. The diameter of the particles appearing under the field of view were measured until about 200 niosomes were measured individually, and their size range and mean diameter were calculated

Drug content

Proniosomes formulation equivalent to 40 mg of curcumin was taken into a standard volumetric flask. They were lysed with 100 ml of propane-1-ol by shaking and 1 ml of the mixture subsequently diluted with phosphate buffer (pH 7.4). The absorbance was measured spectrophotometrically at 254 nm and drug content calculated from the calibration curve of curcumin in phosphate buffer (pH 7.4).

Scanning electron microscopy (SEM) studies

Pure drug and selected formulations were sputtered coated using pelco gold palladium coaters. The surface morphology of the layered sample was examined using SEM. The samples were 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 which when detected, are used to form images and provides information about the specimens. [18]

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 osmotic 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 9-12 hrs) till the absorbance reached a constant reading indicating no drug is available in unentrapped form. The niosomal suspension in the dialysis tube was further lysed with propane-1-ol and estimated the entrapped drug by UV spectrophotometric method at 254 nm. Entrapment efficiency (E) was calculated using Eq 1 [19].

$$E (\%) = 100(TD - DD)/TD \quad \ldots\ldots\ldots\ldots\ldots (1)$$

where TD is total drug and DD is diffused drug

Measurement of angle of repose

Angle of repose of dry proniosome of curcumin powder was measured by funnel method [20].. Proniosomes powder was poured into a funnel which was fixed at a position so that the 12 mm outlet orifice of the funnel is 5cm above a 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

Rate of hydration (Spontaneity)

Spontaneity of niosomes formation is described as the 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 without agitation and the solution kept for about 15-20 min after which a drop of the aqueous layer was withdrawn and placed on Neubaur's chamber. The number of niosomes formed from proniosomes were counted.

Stability studies

The ability of vesicles to retain the drug (Drug Retention Behaviour) was assessed by keeping the proniosomal gel at three different temperature conditions, i.e., refrigeration (4 – 8 ^{0}C), room (25 ± 2 ^{0}C) and oven (45 ± 2 ^{0}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 studied by determining drug content after storage for one month [21].

In vitro skin permeation study

The *in vitro* rat skin permeation study was carried out as per the guidelines compiled by the Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA), 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 were followed [22]. The abdominal hair of albino rats (wistar strain), weighing 200 ± 20 g, was shaved using a hand razor. Care was taken not to damage the skin surface. The rats were sacrificed by administration of excess chloroform inhalation and the abdominal skin of the rats were 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 reduces 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 upwards into the donor compartment. The donor compartment was filled with the proniosomal gel formulation. Phosphate buffer (15 ml, pH 7.4) containing 10% PEG was used as receptor medium to maintain a sink condition. The available diffusion area of the cell was 3.14 cm^2. The receptor compartment was maintained at 37 ± 1^0C, with a 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. The samples withdrawn from the receptor compartment were then analyzed by using UV spectrophotometer at 254 nm.

Statistical analysis

To ascertain drug release mechanism and release rate, the data were model fitted to various release models - zero order (Eq 2), Higuchi (Eq 3) and Korsmeyer-Peppas (Eq 4) - using PCP Disso V3.0 dissolution software [23,24].

Release rate = k …… (2)

Release rate = $kt^{0.5}$ .....(3)

Release = kt^n ……(4)

where k is aconstant, *n* is the release exponent

RESULTS

The low cost, greater stability and resultant ease of storage of nonionic surfactant vesicles have lead to the exploitation of these vesicles as alternatives to phospholipids vesicles for the enhancement of dermal and/or transdermal bioavailability of drugs and substances [25].

SEM (Fig 1) shows that the niosomes prepared by ether injection method were small in size, unilamellar and spherical in shape with a smooth surface.

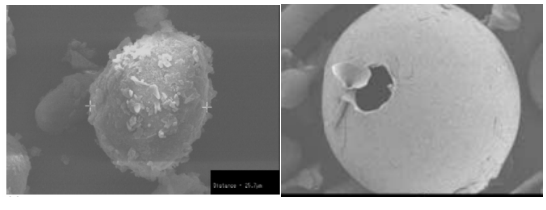

Figure 1: SEM of (a) pure curcumin and (b) formulation PG1 proniosomes

The results of the characterization of the curcumin proniosome formulations are shown in Table 2.

Table 2: Some physicochemical characteristics of curcumin proniosomal formulations

Formulation code	Entrapment Efficiency (%)	Mean particle size (µm, ±SD	Angle of Repose (°)*	Drug content (%)*	Rate of spontaneity Mm^3x10^3
PG1	82.30±0.48	3.84±0.35	36.22±0.43	89±0.42	11.19±0.35
PG2	83.40±0.76	4.66±0.35	37°.22±0.18	85±0.20	15.25±0.58
PG3	85.20±0.59	4.25±0.35	34°.22±0.23	82±0.83	14.54±0.9
PG4	86.80±0.62	4.32±0.35	33°.22±0.06	84±0.55	16.58±0.75

The proniosomes were in the size range 3.84 - 4.66 µm. Entrapment efficiency was 82.3 % for PG1whereas it was 83.4, 85.2%, and 86.8, for formulations PG2, PG3 and PG4, respectively. Angle of repose of the formulation was between 33.2 and 36.2^0. Drug content was highest in formulation PG1 containing (89 %). Rate of spontaneity was between 11.19 and 16.58. Stability data for formulation PG4 showed that drug content, after storage under various temperature conditions was 99.5 % (refrigerated condition), 99.2 % (room temperature) and 93.0 % (oven temperatures).

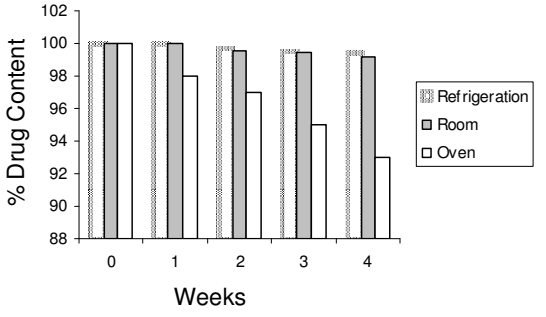

Figure 2: Stability of formulation PG4 under different conditions

Maximum *in vitro* skin release in 24 h was shown by formulation PG4 (78.5 %) and minimum (61.8 %) by formulation PG1.

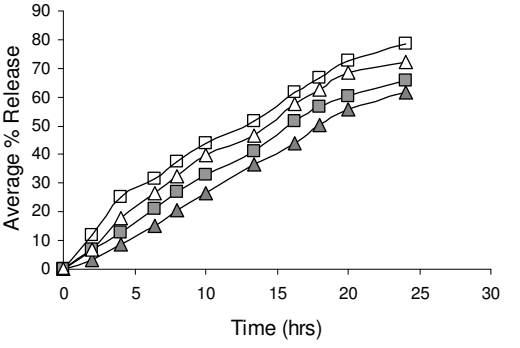

Figure 3: Cumulative curcumin permeation through rat abdominal skin from different curcumin niosome formulations (***Key:*** ▲= PG1; ■ PG2; ∆ = PG3; □ = PG4)

Kinetic parameters determined are shown in Table 3.

To ascertain drug release mechanism and release rate, the release data were fittedinto release models using PCP Disso V3.0 dissolution software. The models selected were zero order, Higuchi matrix and Korsmeyer-Peppas.

DISCUSSION

The objective in developing proniosomes was to devise a method of producing a nonionic surfactant based dosage form at the point of

Table 3: Drug release kinetic parameters for the formulations

Formulation	Zero order		Higuchi		Korsmeyer-Peppas		
	R	*K*	*R*	*K*	*Slope (n)*	*R*	*K*
PG1	0.8461	1.2355	0.9825	10.0458	0.7562	0.9752	5.0038
PG2	0.8124	1.0832	0.9755	8.8554	0.6658	0.9521	5.7985
PG3	0.9052	1.3568	0.9935	10.1652	0.6521	0.9842	4.8856
PG4	0.9121	1.4881	0.9928	10.1045	0.6843	0.9621	6.2541

use to avoid problems of physical and chemical instability found in storage of some surfactant-based dosage forms. By creating a dry formulation, issues related to hydrolysis of the active ingredient or surfactants are avoided.

Curcumin is lipid soluble so dissolved in chloroform and dispersed uniformly throughout the composition. From the above study it was observed that as the cholesterol content in the vesicles increased, the incorporation of the drug in the vesicles also increased. Cholesterol is the known to increase the rigidity of the proniosomal membrane.

The order of encapsulation efficiency increases when the concentration of span-80 was increased. In all the niosomes prepared with spans, as the concentration of surfactant increased drug entrapment efficiency increased. The encapsulation efficiency of niosomes is governed by the ability of formulation to retain drug molecules in the aqueous core or in the bilayer membrane of the vesicles. Cholesterol improves the fluidity of the bilayer membrane and improves the stability of bilayer membrane in the presence of biological fluids such as blood/plasma. This characteristic of cholesterol decreasing leakage of bilayer structure and producing surface smoothness diminish at higher proportions as it imparts crystallinity to the bilayer

The amount of drug retained within the vesicles under defined conditions ultimately governs the shelf life of the drug. The results showed that proniosomal gel formulation was quite stable at refrigeration and room temperatures as not much leakage of drug was found at these temperatures (Figure 2). Percent drug retained at 45^0C might have decreased due to the melting of the surfactant and lipid present in the formulation. Therefore, the proniosomal gel formulations can be stored at either refrigeration or room temperature. *In vitro* drug release studies (figure 3) indicate that in formulation PG1, 76% of drug was released after 24 hours while PG3 and PG4 had lesser drug release after 24 hours. This showed that cholesterol formed a film around the vesicles and increased the micro viscosity of the bi-layer. The thickness of the film depends upon the concentration of cholesterol. Hence, inclusion of cholesterol improved drug retention time and thus reduced permeability. The value of n in Korsmeyer-Peppas model is less than 1, indicating Fickian diffusion and anomalous transport.

It is necessary to improve the bioavailability of curcumin in order to fully utilize the potential of this agent, and therefore a growing number of research groups are working on this aim. The study conducted by Li *et al* [24] investigated the effect of liposomal curcumin on pancreatic carcinoma cells and suppression of KF - kB activity [26]. The incorporated curcumin in liposome's showed a dose related increase in apoptosis of carcinoma cells and suppression of NF- B activity. The study conducted by Kunwar *et al* compared the cellular uptake of liposomal and albumin-loaded curcumin by the splenetic lymphocytes and EL4 lymphoma cells [27].

CONCLUSION

Proniosomal systems of curcumin made with Span 80 exhibited good physicochemical and release properties and can easily be prepared. Formulations prepared using Span 80 with cholesterol showed promising results. Proniosomes may be a promising carrier for curcumin and other drugs, especially due to their simple production and facile scale up. It is recommended that proniosomal formulations should be kept refrigerated to achieve best stability. The in-vitro permeation of curcumin from proniosomes of various compositions and types of nonionic surfactants have been studied and evaluated. However, *in-vivo* studies are required to prove their actual utility as vehicles for transdermal drug delivery.

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