IJAER/ July-August -2022/Volume-11/Issue-4 ISSN: 2278-9677
International Journal of Arts & Education Research

PREPARATION AND EVALUATION OF BOSWELLIC ACID LOADED TRANSFEROSOME FOR TOPICAL DELIVERY

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ABSTRACT

Transfersomes are particularly ultradeformable (ultraflexible) lipid supramolecular aggregates, which use to penetrate the mammalian skin. Transfersome is a type of formulation carrier system which is capable of transdermal delivery of low as well as high molecular weight drugs. Transfersomes penetrate through the pores of stratum corneum of skin which are smaller than its size and get into the underlying viable skin in intact form.Arthiritis is a disease in which drug penentration through skin is the main point of interest which is fulfilled by the transferosome. But, stratum corneum forms the most formidable barrier for the penetration of drug through skin. To overcome the stratum corneum barrier, the use of lipid vesicles like transfersomes in delivery systems has involved increasing attention in recent years. The aim of the present study was to statistically optimize the vesicular formulations (Transfersomes) for enhanced skin delivery of a model drug Boswellic acid for treatment of arthiritis.

Key words: Transferosomes.

INTRODUCTION

An efficacious, successful therapeutic treatment cannot be achieved in most cases, often due to many reasons, such as the occurrence of hepatic first-pass metabolism, adverse side effects, the rejection of invasive treatments and poor patient compliance [1]. Therefore, several drug delivery systems have been developed and studied over the past decades to overcome these problems. One promising approach is the use of transdermal delivery systems, as they are minimally invasive methods without first-pass effects. However, the barrier function of the skin that prevents or dampens the transdermal delivery of therapeutic agents has to be addressed [2,3]. Nanoencapsulation using a lipid based vesicular system such as liposomes has been used to overcome the aforesaid challenge [4]. Liposomes facilitate drug transport through the skin by three possible mechanisms: adsorption to the skin surface with a subsequent transferring of the drug directly from vesicles to skin, fusion with the lipid matrix of the stratum corneum, thereby increasing drug partitioning into the skin, and lipid exchange between the liposomal membrane and cell membrane, facilitating the diffusion of the drug across the membrane [5,6]. However, the problem with conventional liposomes is that they do not deeply penetrate into the viable skin and blood circulation [7–9]. Therefore, liposomes have been widely used as drug carriers for

dermal delivery and not for transdermal delivery. Conventional liposomes also have limitations, such as the poor encapsulation effciency of hydrophilic drugs, an unstable membrane that results in leaky behavior and a short half-life [10–12]. These major obstacles have led to the discovery and development of other novel vesicles such as niosomes, sphingosomes, bilosomes, chitosomes, transfersomes, ethosomes and invasomes. Niosomes were first reported in the early 1970s. They are composed of nonionic surfactants (i.e., of alkyl or di-alkyl polyglycerol ether class, alkyl ethers, alkyl esters or alkyl amides); cholesterol and, sometimes, ionic amphiphiles. The cholesterol provides rigidity to the vesicular bilayer, whereas nonionic surfactants increase the size and entrapment efficiency of niosomes [13]. Furthermore, some ionic amphiphiles such as dicetyl phosphate (negatively charged molecule) and stearylamine (positively charged molecule) are used in the niosomes for enhancing the entrapment efficiency, efficacy and stability [14]. Moreover, they are identified as a better drug carrier system over liposomes due to considering factors such as a high chemical stability, high bioavailability, high entrapment efficiency and being inexpensive. According to the literature, niosomes tend to enhance the residence time of therapeutic drugs in the stratum corneum and epidermis, meanwhile reducing the systemic drug absorption and thereby improving the trapped drug penetration across the skin [15]. Chitosomes, liposomes-based vesicles coated with chitosan polymer, were first described in the early 1990s. Chitosan is used to improve the vesicular stability by modifying the surface properties of liposomes [16]. Moreover, chitosan provided mucoadhesive properties to the liposomes [17]. As mentioned by Mertins and Dimova in 2011, chitosan increases the membrane structural integrity of liposomes, decreases the membrane fluidity and thereby enhances the physicochemical stability of liposomes [18]. Sphingosomes were first introduced in the late 1990s. They can be identified as liposomes made from sphingolipids (such as lysoglycosphingolipids, hexadecasphinganine, n acylsphingosines, phosphoglycosphingolipids and gangliosides) and cholesterol. Sphingosomes are more stable than phospholipid liposomes, because sphingolipids consist of only amide and ether linkages that are more hydrolysis-resistant than that of lecithin ester linkages. They also contain fewer double bonds than lecithin and, therefore, are less subjected to rancidity [19].

The Burseraceae family consists of 17 genera and 500–600 species, which are either trees or shrubs, often spiny. Many of them contain latex, gum-resins or oils, which can be strongly aromatic. This family is widespread in all tropical regions, extending into the subtropics, and is often a dominant constituent of the vegetation in dry lowland Eastern African areas. The resins of several species of this family are of considerable commercial value as raw material of incense, balm and myrrh. *Boswellia* species are trees or shrubs. The genus *Boswellia* Roxb. Ex Colber (1807) is centered in North-East Africa, where about 75% of the species are endemic. About 20 species are known in the dry regions of tropical Africa and in India, and one species is found in Madagascar. [20]

MATERIAL AND METHOD

Materials

Active Ingredient

Boswellic acid grade quality with (PDR-No. 30076166/Lot no.: SB1W0030) was received from MSN Labs. from India. It is a white crystalline powder with a characteristic odor, mean particle size of about 25 μm and melting range of 184-186°C. This sample meets the current Indian Pharmacopeia (IP) and United State Pharmacopoeia (USP).

Inactive ingredients

Tween 80 and Span 80 from Loba Chem Pvt. Ltd, Mumbai, Lecithin and Triton x-100 from IMEDIA Laboratories Pvt. Ltd, Mumbai, 6-carboxyfluorescein, Acros, Organics, New Delhi. All other ingredients used were of analytical grade.

Analytical Reagents

Sodium hydroxide, potassium phosphate monobasic, acetonitrile (ACN), triethylamine and orthophosphoric acid were procured from Central Drug House (New Delhi, INDIA). All reagents used were of analytical grade.

Methods

Preparation of Transfersome:

The transfersome were prepared by modified hand shaking, lipid film hydration technique. The composition of formulation is in (Table a). Drug, lecithin (PC) and edge activator were dissolved in ethanol:chloroform (1:1) mixture. Organic solvent was removed by evaporation while hand shaking above lipid transition temperature (43°C). A thin lipid film was formed inside the flask wall with rotation. The thin film was kept overnight for complete evaporation of solvent. The film was then hydrated with phosphate buffer (pH 7.4) with gentle shaking for 15 minute at corresponding temperature. The transfersome suspension further hydrated up to 1 hour at 2-80 C [3,4].

Optimization of formulation:

There are various process variables which could affect the preparation and properties of the transfersomes. The preparation procedure was accordingly optimized and validated. The preparation of

transfersomes containing boswellic acid involves various process variables such as effect of lecithin:surfactant ratio (95:05, 85:15), effect of various solvents (ethanol, isopropyl alcohol) and effect of various surfactants (Span80, Tween80), optimization was done by selecting entrapment efficiency of drug. During the preparation of a particular system, the other variables were kept constant.

Interference Study:

Interference study was carried out for any interference of drug-polymer, drug diluents and drug-lubricant used in the formulation. The interference study was carried using FTIR.

Characterization of Transfersomes:

The Morphological characterization of transfersome vesicle such as shape and surface feature were projected by Photomicroscopy using a digital Labomed camera in 40x resolution and transmission electron microscopy using (TEM, FEI Philips Tecnai 12) and photomicrograph was taken [5]. Chemical characterization includes those studies which established the purity and potency of various transfersomal constitutions.

Determination of Entrapment efficiency percentage:

Transfersome entrapped boswellic acid was estimated by centrifugation method. The prepared transfersome were placed in centrifugation tube and centrifuged at 14000 rpm for 30 minute. The supernatant (1ml) was withdrawn and diluted with phosphate buffer (pH 7.4). The unentrapped boswellic acid was determined by UV spectrophotometer at 427.2nm. The samples from the supernatant were diluted 100 times before going for absorbance measurement. The free boswellic acid in the supernatant gives us the total amount of unentrapped drug. Encapsulation efficiency is expressed as the percent of drug trapped.

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

Further, after fusing the vesicle with Triton X-100 and amount of trapped drug was estimated by UV spectrophotometer with suitable dilution by Phosphate buffer (pH 7.4). The vesicle was washed with firstly phosphate buffer (pH 7.4) and 3-4 times with distilled water and suspended in distilled water [6,7].

Vesicle size, size distribution and zeta potential analysis:

The average diameter and size distribution profile and zeta potential analysis of vesicles were determined by Malvern Zetasizer DTS version 5.03 (Malvern, UK). Zeta potential was analyzed to measure the permeation of transfersome by studying its colloidal property and stability of the vesicle [4-7].

***Preparation of topical hydrogel* :**

As a vehicle for incorporation of transfersomes for topical delivery, carbopol hydrogels were prepared. Optimized boswellic acid transfersomes aqueous dispersion was utilized for the formulation of topical hydrogel (Table b). Optimized transfersome dispersion equivalent to 200 mg of pure drug was taken. Hydrogel polymer such as carbopol 971P was utilized to obtain controlled release topical hydrogel. The appropriate quantity of carbopol 971P powder (0.5, 1,1.5, 2 g) was dispersed into vigorously stirred (stirred by magnetic stirrer) distilled water (taking care to avoid the formation of in dispersible lumps) and allowed to hydrate for 4 to 5 hrs. The dispersion was neutralized with 10% (w/v) aqueous solution of sodium hydroxide to adjust the pH [8].

Evaluation of Topical Hydrogel:

Determination of pH:

The value of pH of topical hydrogels was measured by using digital pH meter (ELICO.LI 610 pH meter) at the room temperature.

***In vitro* Skin permeation Studies:**

Modified Franz diffusion cell with a receiver compartment volume of 50ml and effective diffusion area of 2.50cm^2 was used for this study. *In vitro* drug study was performed by using goat skin in phosphate buffer solution (pH 7.4). Fresh Abdominal skin of goat were collected from slaughterhouse and used in the permeation experiments. Abdominal skin hairs were removed and the skin was hydrated in normal saline solution. The adipose tissue layer of the skin was removed by rubbing with a cotton swab. Skin was kept in isopropyl alcohol solution and stored at 0-4^{0}C [9]. To perform skin permeation study, treated skin was mounted horizontally on the receptor compartment with the stratum corneum side facing upwards towards the donor compartment of Franz diffusion cell. The effective permeation area of donor compartment exposed to receptor compartment was 2.50cm^2 and capacity of receptor compartment was 50ml. The receptor compartment was filled with 50ml of phosphate buffer (pH 7.4) saline maintained at 37± 0.5^{o}C and stirred by a magnetic bar at 100rpm. Hydrogel formulation (equivalent to 10mg drug) was placed on the skin and the top of the diffusion cell was covered. At appropriate time intervals 1 ml

aliquots of the receptor medium were withdrawn and immediately replaced by an equal volume of freshphosphate buffers (pH 7.4) to maintain sink conditions. Correction factors for each aliquot were considered in calculation of release profile. The samples were analyzed spectrophotometrically at λ max 427.2nm Fig 1. [10-12].

Skin deposition studies of optimized formulation:

At the end of the permeation experiments (after 24hr), the skin surface was washed five times with ethanol: PBS pH 7.4 (1:1), then with water to remove excess drug from surface. The skin was then cut into small pieces. The tissue was further homogenized with ethanol: buffer solution pH 7.4 (1:1) and left for 6hr at room temperature. After shaking for 5 minutes and centrifuging for 5 minutes at 5000rpm, the boswellic acid content was analyzed by UV visible spectrophotometric method after appropriate dilutions with Phosphate buffer solution (pH 7.4) at 427.2nm. The result was compared with the control group using *student's t-test.*

Table A: Composition of formulation

Edge activator	Formulation code	LC:TS*	Solvent
TWEEN 80	T-1	95:05	ETHANOL
	T-2	85:15	
	T-3	95:05	ISOPROPYL ALCOHOL
	T-4	85:15	
SPAN 80	T-5	95:05	ETHANOL
	T-6	85:15	
	T-7	95:05	ISOPROPYL ALCOHOL
	T-8	85:15	

***LC indicates Lacithin and TS indicates Tween 80 and Span 80**

Table B: Formulation of topical hydrogel

Hydrogel ingredients	Topical Hydrogel formulations			
	H1	H2	H3	H4
Transfersome	Eqv. To2% of drug	Eqv. To2% of drug	Eqv. To2% of drug	Eqv. To2% of drug
Carbopol971P	0.50%	1.00%	1.50%	2.00%
Propylene glycol	10%	10%	10%	10%
Glycerol	30%	30%	30%	30%
Distilled water	Quantity sufficient			

Table C: Entrapment efficiency of different formulation

Molar ratio (LC:TS)*	Formulation Code	Entrapment efficiency*(%)
Tween 80		
95:05	T1	68.2±0.074
85:15	T2	71.6±0.021
95:05	T3	73.3±0.018
85:15	T4	76.3±0.086
Span 80		
95:05	T5	73.6±0.033
85:15	T6	78.9±0.045
95:05	T7	81.5±0.012
85:15	T8	89.6 ±0.049

***All values are mean ± S.D. for n=3, LC indicates Lecithin, TS indicates Tween 80 & Span 80**

RESULT AND DISCUSSION

Transfersomes were used for non-invasive delivery of drugs into or across the skin. Transfersomes are also known as elastic liposomes or flexible vesicles which have better penetration ability than conventional liposomes. So in present vocation deformable lipid vesicles 'Transfersomes' were formulated. The proposed system is more stable, having higher entrapment efficiency, can be used as self penetration enhancer, easy to scale up & better for dermal delivery.

Characterization of transfersomes:

The surface morphology was studied by Optical Microscopy and transmission electron microscopy. The results were shown in the Fig 2 & 3.

Interference Study:

Interference study was carried out for any interference of drug-polymer, drug diluents and drug-lubricant used in the formulation, which reveals that there is no interaction between drug and polymer used in formulation Fig 4.

Entrapment Efficiency:

The % entrapment efficiency of deformable vesicles formulations were found to be in the range of 68.2±0.074 to 89.6±0.049 (Table c). Entrapment efficiency of the T8 formulation was high maximum 89.6±0.049 for T8) because of the increase in the ratio of lipid volume in the vesicles as compared to the encapsulated aqueous volume. The effect of phospholipids and edge activator ratio in the lipid components of vesicles on the entrapment efficiency of lipophilic drug, boswellic acid, the efficiency decreased with increasing surfactant concentration and thus increased with increasing PC concentration but have certain limit of PC:EA.

Vesicle size, size distribution and surface charge (zeta potential):

The vesicle size, size distribution and zeta potential were determined by light scattering method by Malvern Zetasizer (DTS version 5.03, Malvern, UK) of the optimized boswellic acid loaded formulation (T8). The mean vesicle diameter was found to be 339.3 nm Fig 5 & 6. Size distribution curve confirms the normal size distribution of the vesicles. Transfersomal vesicles containing ethanol were larger in size compared with vesicles contained IPA because ethanol have greater solubility with water. The effect of edge activators on the size of vesicles that Span80 was small in size but no significant difference with Tween80 when solvent system was similar.

pH value of topical hydrogel:

The value of pH of topical hydrogels was measured by using digital pH meter (ELICO.LI 610 pH meter) at the room temperature. The value of pH of topical hydrogels H1, H2, H3 and H4 were found 6.6±0.12, 6.4±0.14, 6.6±0.02 and 6.8±0.25 respectively.

***In-vitro* release study of topical hydrogel:**

The dissolution rate studies for each of the formulations were performed in order to assess the effect of change in surfactant concentration on release profile. *In vitro* release study of topical hydrogel of boswellic acid transfersomes were carried out for 24 hours by using modified Franz diffusion cells in (pH 7.4) phosphate

buffer maintained at 37±0.5oC temperature and stirred by a magnetic bar at 100rpm under sink condition and the dissolution profile of all hydrogel formulations obtained from the dissolution data were shown in (Table d) and (figure 7). The result of dissolution profiles showed that the concentration of gelling agent (Carbopol 971P) in the range of 0.5% to 2.0% affects the release rate slightly. Regarding the formulation H1 to H4, the values of drug release after 24 hr were found to be 50.61%, 49.11%, 48.89% and 48.54% respectively. The values of drug release of the formulations H1 to H4 after 4 hour were found to be 20.81%, 20.21%, 19.64% and 19.31% respectively. This result of dissolution profile showed slight initial burst release. This is probably caused by the release of drug absorbed on the transfersome surface or precipitated from the superficial lipid layer. Prolonged release in the later stage can be attributed to the slow diffusion of the drug from the lipid vesicle.

Kinetic Analysis of Dissolution Data:

The drug release data were explored for the type of release mechanism followed. Release kinetic study of all formulation (H1 to H4) was studied (Table e) for different kinetic equation (zero order, first order and Higuchi equation). The best fit with higher correlation ($r2 > 0.99$) was found with the Higuchi's equation for all the formulations, which means that release of boswellic acid from the lipid ilayer vesicles were due to diffusion. Release kinetic study was again verified by putting the values of release data in modern biopharmaceutics software MB-V6 and found that all the formulations follows Higuchi model (Table f). Hence, we can state that release of boswellic acid from the lipid bilayer system was mainly due to diffusion mechanism.

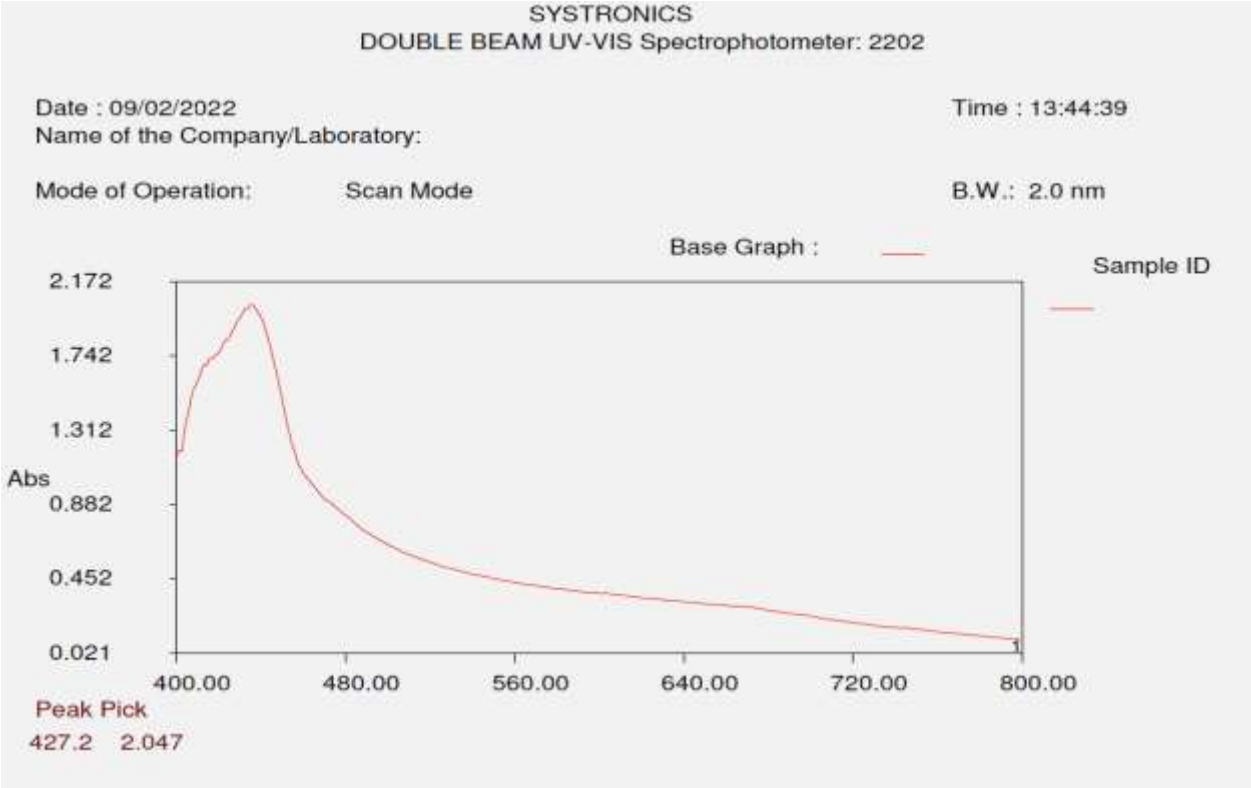

Fig. 1: Determination of absorption maxima (λmax) in PBS (pH 7.4)

Fig. 2: Optical photomicrograph of Boswellic acid loaded transfersome at 10×

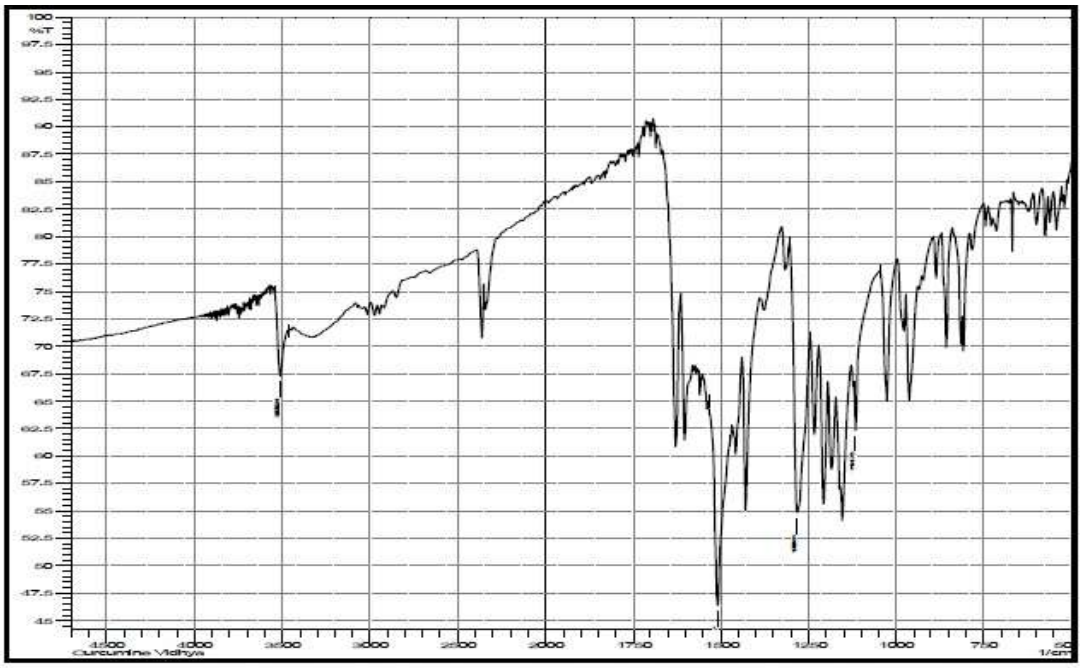

FIGURE 3 IR SPECTRA OF BOSWELLIC ACID WITH POLYMER SAMPLE

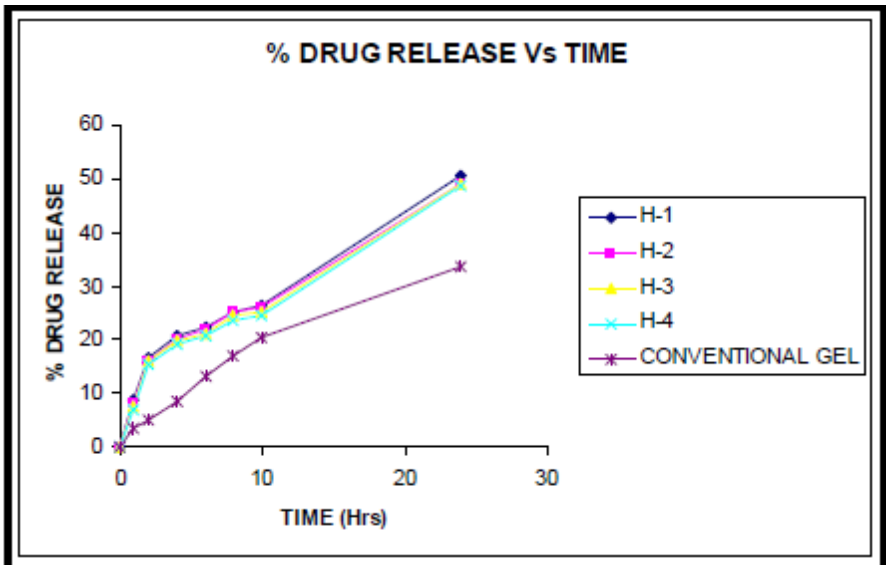

FIGURE 4 DRUG RELEASE STUDY OF TOPICAL HYDROGEL

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