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TRANSFERSOME FOR TREATMENT OF HERPES ZOOSTER OF ANTIVIRAL DRUGS

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ABSTRACT

Transfersome is a very versatile, push responsive and multifaceted total. Its favored shape is a ultra deformable vesicle having a watery center encompassed by the mind boggling lipid bi-layer. The name signifies "conveying body", and is duplicated from the Latin word 'transferre', signifying 'to convey over', and the Greek word 'soma', for a 'body. A Transfersome transporter is a manufactured vesicle and takes after the characteristic cell vesicle. In this way it is appropriate for focused and controlled medication delivery. Transfersomes are vesicles, which are self-improved totals with ultra-adaptable layer. These vesicular transfersomes are more flexible than the standard liposomes and in this manner appropriate for the skin penetration.[2] Transfersomes are advantageous as phospholipids vesicles for transdermal drug delivery. Because of their self-optimized and ultra flexible membrane properties, they are able to deliver the drug reproducibly either into or through the skin, depending on the choice of administration or application, with high efficiency. The vesicular transfersomes are more elastic than the standard Transfersomes and thus well suited for the skin penetration. Transfersomes overcome the skin penetration difficulty by squeezing themselves along the intracellular sealing lipid of the stratum corneum Skin permeation profile of acyclovir through transfersomes formulations was observed in the present study. The investigations revealed an enhanced transdermal delivery of acyclovir. Hence, it could be concluded that transfersomes may be suitable carrier for transdermal delivery of acyclovir.

KEYWORDS: Transfersomes (Vesicular System), TDDS, Skin Penetration, Acyclovir, Gel permeability, Liposomes, FTIR, Entrapment.

INTRODUCTION

Transfersomes (vesicular system)

The term Transfersome and the basic model were presented in 1991 by Gregor Cevc. From that point forward, tremendous measure of research is going on worldwide on these versatile vesicles under different titles like adaptable vesicles, ethosomes, and so forth. In broadest sense, a Transfersome is a very versatile, push responsive and multifaceted total. Its favored shape is a ultra deformable vesicle having a watery center encompassed by the mind boggling lipid bilayer. Transfersome is a term enlisted as a trademark by the German organization IDEA AG, and utilized by it to allude to its restrictive medication conveyance innovation. The name signifies "conveying body", and is duplicated from the Latin word 'transferre', signifying 'to convey over', and the Greek word 'soma', for a 'body'. A Transfersome transporter is a manufactured vesicle and takes after the characteristic cell vesicle. In this way it is appropriate for focused and controlled medication delivery.[1] Transfersomes are vesicles, which are self-improved totals with ultra-adaptable layer. These vesicular transfersomes are more flexible than the standard liposomes and in this manner appropriate for the skin penetration.[2]

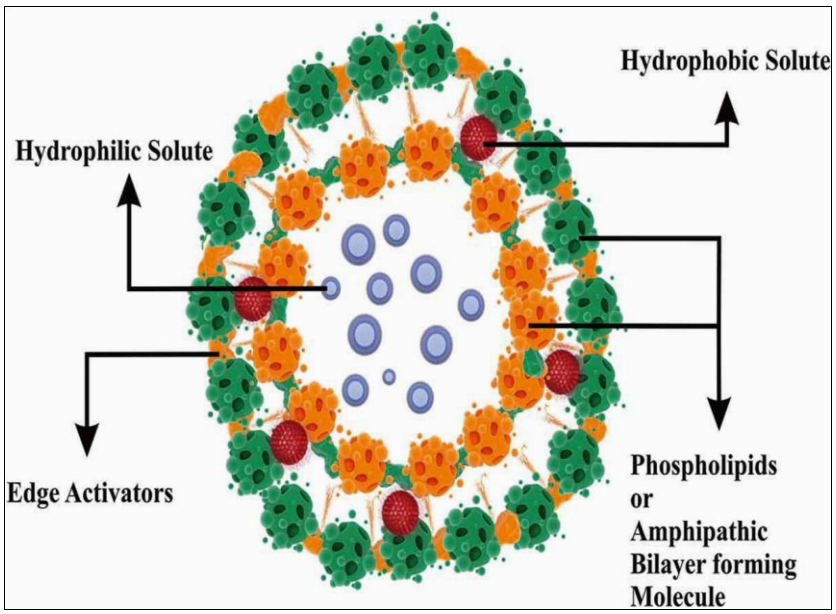

Figure 1: Structure of Transfersomes.

Rationale for selecting the lipid vesicles (Transfersomes) as a TDDS

There are various occurrences where the most appropriate medication admission strategies, similar to oral course, were not doable and elective courses must be looked for. However, intravenous organization of the medicament keeps away from a considerable lot of these

setbacks, (for example, gastrointestinal and hepatic digestion), its intrusive and fearful nature (especially for ceaseless organization) has empowered the look for elective methodologies. Transdermal Topical medication conveyance offers a few particular points of interest including nearly expansive and fipromptly accessible surface zone for assimilation, simplicity of utilization and end of treatment.

- Transfersomes are amphiphilic in nature so able to accommodate both hydrophilic as well as lipophilic drugs.
- Transfersomes release the drug in a continued manner for a prolonged period of time at a predetermined rate.
- Transfersomes can distort and pass through narrow constriction (from 5-10 times less than their own diameter) without measurable loss.
- Transfersomes can act as a carrier for low and high molecular weight drugs.
- Transfersomes have high entrapment efficiency.
- Transfersomes are used for both, pertinent and systemic delivery of drugs.
- They protect the encapsulated drug from metabolic degradation.[3]

Advantages

1. They can encapsulate both hydrophilic and lipophilic moieties.
2. Prolong half-lives of drugs by increasing duration in systemic circulation due to encapsulation.
3. Ability to target organs for drug delivery.
4. Biodegradability and lack of toxicity[4]

Scope of Transfersome

Transfersome innovation is most appropriate for non-obtrusive conveyance of helpful atoms crosswise over open natural boundaries. The Transfersome vesicles can transport over the skin, for ex, atoms that are too huge to diffuse through the hindrance. Ex. incorporates fundamental conveyance of remedially significant measures of macromolecules, for example, insulin or interferon, crosswise over in place mammalian skin. Other reason incorporates the vehicle of little particle drugs which have certain physicochemical properties which would some way or another stay away from them from diffusing over the hindrance. Exchange some gear is the bearer's capacity to target fringe, subcutaneous tissue. This capacity depends on minimisation of the bearer associated sedate leeway through cutaneous veins plexus, the non-fenestrated blood slim dividers in the skin together with the tight intersections between

endothelial cells block vesicles getting specifically into blood, accordingly boosting neighborhood medicate maintenance and affinity to achieve the fringe tissue targets.[5]

Limitations of Transfersomes

- Chemically unstable
- Expensive
- Less purity of phospholipids.
- Predisposition to oxidative degradation.[6]

Necessity of transfersomes for skin delivery

Transfersomes are phospholipids vesicles for transdermal drug delivery. Because of their self-optimized and ultra-felxible membrane properties, they are able to deliver the drug reproducibly either into or through the skin, depending on the choice of administration or application, with the high efficiency. The vesicular transfersome are more elastic than the standard liposome and thus well the standard liposomes and thus well suited for the skin penetration difficulty by squeezing themselves along in the intracellular sealing lipid of the stratum corneum.[7]

Silent features of Transfersomes

Transfersomes possess an infrastructure consisting of hydrophilic moieties together and as a result can accommodate drug molecules with wide range of solubility as show in fig 1. Transfersomes can deform and pass through narrow constriction (from 5to 10 times less than their own diameter) without measurable loss. This high deformability gives better penetration of intact vesicles. They can act as a carrier for low as well as high molecular weight drugs e.g. analgesic, anaesthetic, corticosteroids, sex hormone, anticancer, insulin, gap junction protein, and albumin. They are biocompatible and biodegradable as they are made from natural phospholipids similar to liposome. They have high entrapment efficiency, in case of lipophilic drug near to 90%. They protect the encapsulated drug from metabolic degradation. They act as depot, releasing their contect slowly and gradually. They can be used for both systemic as well as topical delivery of drug. Esay to scale up, as procedure is simple, do not involve lenghthy procedure and unnecessary use of pharmaceutically unacceptable additives.[8]

Table 1: Different Drugs Used and Results Obtained of Different Studies of Transfersomes for transdermal application.[9]

S. No.	System	Drug	Results
1.	Transfersomes	Insulin	High entrapment efficiency, Improved transdermal flux.
2.	Transfersomes	Interferon-α	Vaccine
3.	Transfersomes	Interleukin-2	Controlled release, reduce stability problem
4.	Transfersomes	Soluble proteins	Permits, noninvasive immunization.
5.	Transfersomes	Hydrocortisne Dexamethasone	Increased biological potency, Prolonged effect, Reduced dosage
6.	Transfersomes	Triamcinolone acetonide	Both for local and systemic delivery
7.	Transfersomes	Diclofenac Tetracaine Lidocaine	Non-invasive treatment of local pain on direct topical application
8.	Transfersomes	Oestradiol	Improved transdermal flux
9.	Transfersomes	Tamoxifen	Improved transdermal flux
10.	Elastic liposome	Zidovudine	Sustained drug delivery
11.	Transfersomes	Vaccine	Both for Local and Systemic delivery

Table 2: Composition for Transfersome Formulations.[9]

Class	Example	Uses
Surfactants	Sodiumcholate,sodium desoxycholate,Span60,Tween-60,tween-80	Flexibility provider
Phospholipids	Dipalmitylphosphatidylcholine, Distearylphosphatidycholine,egg phosphatidycholine, soya phosphatidyl choline, lecithin	Vesicle providing agents
Solvents	Ethanol,methanol, Chloroform	Solvent
Buffering agents	Saline phosphate buffer(ph 6.4)	Hydration medium
Dye	Fluorescein-DHPE, Nile-red,Rhodamine-DHPH,rhodamine-123	For conofocal laser Micoroscopy study

Polymers employed for the development of transferosomes[10]

1) Lecithin[10]

It is a yellow-brownish fatty substances occurring in animal and plant tissues composed of phosphoric acid, choline, fatty acids, glycerol, glycolipids, triglycerides and phospholipids. It was first isolated in 1846 by the French chemist and pharmacist Theodore gobley. He originated lecithin from egg yolk. Established chemical formula of phosphatidyl choline. Available from sources of soybean, milk, marine sources, rapeseed, cottonseed and

sunflower. Phosphatidylcholine dissolve in ethanol. Lecithin is a source of choline an essential nutrient. It can be totally metabolized by humans.[10]

Applications

- It acts as wetting, stabilizing, choline enrichment carrier.
- Good dispersing agent
- Helps in emulsification and encapsulation
- Acts as catalyst, colour intensifying agent. Good stabilizing and suspending agent. Eliminates foam in water based paints.
- Used as an anti sludge additive in motor lubricants. Anti gumming agent in gasoline, spreading agent, textile, rubber industries.

2) SOYAPHOSPHATIDYL CHOLINE

Phosphatidyl cholines are a class of phospholipid that incorporates choline as a head group. They are major component of biological membranes and can be easily obtained. Phosphatidyl choline is a major constituent of cell membranes and pulmonary surfactant is more commonly found in outer leaflet of a cell membrane, transported between membranes by phosphatidylcholine transfer protein. Phospholipid composed of choline head group and glycerophoric acid with fatty acids. Phospholipase decatalyzes the phosphatidyl choline to form phosphatidic acid and releasing the soluble choline head group into the cytosol. Phosphatidyl choline supplementation slows down aging related processes and improves brain functioning and memory but does not benefit in the patients in dementia.[10]

Uses

Used in the cure of inflammatory bowel disease.

3) DISTEROYL PHOSPHATIDYL CHOLINE[10]

It appears like a solid substance and stable. Incompatible with strong oxidizing agents. Vesicles formed by sonication of saturated chain phosphatidyl cholines in aqueous media have been used extensively as a model for the lipid component in the plasma membrane. The rate of loss of small vesicles and information about the structures to which the small vesicles are converted can be obtained from sedimentation velocity experiments. The kinetic behavior of small disteroyl phosphatidyl choline vesicles is examined. Small single bilayer vesicles are unstable at all temperatures. The vesicles size distributions changed as a function of time at

all temperatures below the phase transition temperatures but constant at transition temperature and above. It is to be stored at -20°C.

4) Dipalmitoyl phosphatidylcholine[10]

It is a phospholipid consisting of two palmitic acids. It is the major constituent of pulmonary surfactant. It is synthesized mainly through remodeling of phosphatidyl choline. 1, 2-dipalmitoyl-sn-glycero-3-PC is a zwitterionic phosphoglyceride that can be used for the preparation of liposomal monolayer. The extent of incorporation of the enzyme glutamyl transpeptidase in erythrocyte membranes was five times higher when proteoliposomes were prepared from L-DPPC as compared to control. L-DPPC incorporated vesicles have potential in establishing active immunotherapy with the antigens.

5) Cholesterol[10]

Cholesterol is a sterol or modified steroid which is a lipid molecule biosynthesized by animal cells to maintain integrity and fluidity. It enables no need of cell membrane, ability to change its shape, able to move by the animals. Cholesterol serves as precursor for vitamin D, steroid hormones, bile acids, it is the principal sterol synthesized by humans. Most of ingested cholesterol is esterified and poorly absorbed. The cholesterol modulates membrane fluidity over a range of physiological temperatures. The trans confirmation of tetracycline ring decrease fluidity but side chain is rigid and planar.

In neurons a myelin sheath is derived from Schwann cell membrane, providing insulation for many conducting impulses. Cholesterol is slightly soluble in water, insoluble in blood it transported in blood stream through lipoproteins. High levels of cholesterol termed as hypercholesterolemia. Low levels of cholesterol results hypocholesterolemia.

6) Deoxycholic acid[10]

It is also known as deoxycholate, cholanoic acid. deoxycholic acid is one of the metabolic byproducts and secondary bile acids of intestinal bacteria. The two primary bile acids secreted by the liver are cholic acid and chenodeoxycholic acid. Soluble in alcohol and acetic acid. Pure form is in white to crystalline powder form. It functions as detergent and isolating agent for membrane proteins such as “men B”.[28] In china, traditional medicine “Niuhuang” comprises active component DCA used in the treating inflammations and enhances immune system. It can be used as immunostimulant. Lyso phosphatidyl choline acyl transferase plays a critical role in its sysnthesis.

Uses

- Used for research purposes in studying liposomes, lipid bilayers, and biological membranes.
- Used in the production if high density lipoproteins.
- Used in healing of local inflammations.

7) Tween 80

It is a nonionic surfactant and emulsifier used in foods and cosmetics. It is also known as polysorbate 80. It is derived from polyethoxylated sorbitan and oleic acid. It is introduced above CMC. It is amber coloured viscous liquid nonionic surfactant, viscous water soluble yellow liquid. It is an excipient that is used to stabilize aqueous formulations of medications for parentral administrant and emulsifier in the antiarrhythmic amidarone. It is used in some eye drops. it is harmful to people in some eye drops it is not carcinogenic. Soluble in ethanol, cottonseed oil. The cosmetic grade of tween 80 has more impurities than food grade.

Uses

1. As an emulsifier in the preparation of food products.
2. Used to identify the phenotype of strain.
3. Used in ice-cream as it increases the resistance of melting
4. Prevents milk proteins from coating the fat droplets.
5. Used as surfactant in soaps and cosmetics.
6. Used as a solubilizer in mouthwash.
7. It is used as an excipient to stabilize aqueous formulations of parenteral preparations and emulsifier in antiarrhythmic amiodarone.

8) Tween 20

It is a clear yellow to yellow green viscous liquid. It is a surfactant whose stability and relative non toxicity used as a detergent and emulsifier in scientific an pharmaceutical applications. It is a derivative of polyoxyethylene derivative of sorbitan monolaurate.

Uses

1. Used as wetting agent in flavour mouth drops spreads like alcohol and mint flavour.
2. It has a broad sense of application in biotechnical sciences.
3. As a washing agent in immunoassays.
4. Saturate binding sites.

5. Stabilize proteins.
6. Pharmaceutically it is used as excipient to stabilize emulsions and suspensions.
7. It is used to remove stamps from envelopes without harming.

9) Span 20

It also known as sorbiton monolaurate. It is a mixture of partial esters of sorbitol and its mono and dianhydrates with edible lauric acid. It is an amber coloured oily viscous liquid, light cream to tan beads or flakes or a hard, waxy solid with a slight odour. Soluble in hot and cold water.

10) Span 80

Also known as sorbiton oleate. It is a nonionic surfactant induced at CMC. Highest skin permeation can be achieved if we use 10% span 80. Cationic lipid vesicles were developed of mainly of span 80. Microemulsions can be formulated by span 80 as lipophilic linker. When lactobacillus cells care grown in a medium supplemented with span 80 showed viability and acid producing activity. Addition of increased concentrations of span 80 to suspensions caused marked changes in stability. Span 80 served as a coaservating agent in microspheres.

11) Ethanol

Ethanol also called as ethyl alcohol, pure alcohol phytoactive drug. It is a volatile, colourless liquid that has a slight odour. Ethanol is produced by fermenting sugars with yeast Ethanol causes alcohol intoxication. Ethanol's hydroxyl group is able to participate in hydrogen bonding rendering it more viscous and less volatile than polar organic compounds such as propane. Ethanol was first used as lamp fuel in US 1840. It remains a common fuel for spirit lamps. It has a complex mode of action and affects multiple systems in brain.

Uses

1. It is used in scents, thermometer as a solvent.
2. Intended for flavourings, colourings and medicines
3. As motor fuels & fuel additive, rocket fuels.
4. As it dissolvers hydrophobic flavours compounds used as beverages in cooking.

12) Chloroform

It is one of the four chloromethanes. It is a colourless, sweet smelling, dense liquid trihalomethane which is considered as hazardous.it is soluble in water, benzene, acetone,

DMSO, diethyl ether, oils, ethanol. It is estimated that over 90% of atmospheric chloroform is of natural origin. it is produced by brown seaweeds, red seaweeds and green seaweeds. Chloroform can be produced by heating a mixture of chlorine and chloromethane or methane. Dueterated chloroform is an isotope logue of chloroform with single deuterium atom.

Uses

1. The major use of chloroform today is the production of chlorofluoromethane which is a major precursor to tetrafluoroethylene. Chlorofluoromethylene used as popular refrigerant.
2. It is used as a solvent in laboratory as it is unreactive miscible with organic liquids not flammable and conveniently volatile. Longer storage of chloroform in polypropylene containers is not advised.
3. Used as a reagent for dichlorocarbene CCL_2 groups which affects ortho formylation of activated aromated rings. Phenols and aldehydes in riemer tiemann reaction.
4. Used as anaesthetic as it depresses CNS system.
5. It increases the movement of K^+ ions through the potassium channels in nerve cells

Characterizations for transferosomes[10,11,12]

1. Entrapment efficiency

It is expressed as the percentage entrapment of the drug. Entrapment efficiency was determined by centrifugation method, the vesicles were disrupted using triton-100(0.1%) or 50% n-propanol.

Entrapment efficiency = amount entrapped / amount added × 100

2. Vesicle size and size distribution

Analysis of the transferosomes vesicle size before sonication determined by using a micrometer scale. The polydispersibility index measurement was carried out by dynamic light scattering with zetasizer HAS 3000. The samples are sonicated before PDI determination.

3. Turbidity Measurements

The turbidity measurements were diluted with distilled water to give a total lipid concentration of 0.312m. sonicate for 5 min. Measure turbidity at 274 nm with UV visible spectrophotometer.

4. Number of vesicle per cubic mm

It is an important parameter for optimizing the composition and other process variables. Transferosome formulations can be diluted for 5 times with 0.9% sodium chloride solution and studied with optical microscopy.

5. Confocal scanning laser microscopy study

The problems associated with problem of fixation, sectioning and staining. Often misinterpretation can be minimized by confocal scanning laser microscopy. In this technique fluorescence markers are incorporated into formulations and light emitted by these markers are used for the determination of mechanism of penetration across the skin.

6. Degree of deformability

The deformability study is done against the pure water as standard. Transferosome preparations are passed through a large number of pores of known size distributions are noted after each pass by dynamic light scattering measurements.

7. Occlusion Effect

For traditional topical preparations, but also can be determined for elastic vesicles. Hydrotaxis is the movement of water in direction is major driving force for transferosomes, from dry surface to deep regions with water rich area. Due to hydration forces the evaporation of water be prevented from skin surface.

8. *In-vitro* drug release

Is performed for determination of permeation rate. Transfersomes were incubated at 320°C and the samples are taken at different times and free drug is separated by mini-column centrifugation. The amount of drug released is then calculated indirectly from the amount of the drug entrapped at zero time as initial amount.

MATERIALS AND METHODS

Characterization of drug: Acyclovir

Physiochemical Properties of Acyclovir[13,14,15,16]

A) Physical evaluation

It refers to the evaluation by sensory characters-taste, appearance, odor, feel of the drug, etc.

B) Solubility

Solubility of the active drug was determined by taking some quantity of active drug (about 1-2mg) in the test tube separately and added the 5ml of the solvent (water, ethanol, methanol, 0.1N HCL, 0.1N NaOH, Chloroform and 7.4 pH buffer) Shake vigorously and kept for some time. Note the solubility of the drug in various solvents (at room temp).

RESULT OF SOLUBILITY

Table 3: Solubility of Acyclovir.

Solvent used	Acyclovir
Distilled Water	Slightly soluble
0.1 N Hydrochloric acid	Freely soluble
Ethanol	Freely soluble
Methanol	Freely soluble
Chloroform	Soluble
0.1 N NaOH	Slightly Soluble
Phosphate buffer pH 7.2	Soluble

C) Melting point

It is one of the parameters to judge the cleanness of drugs. In case of pure chemicals, melting points are very sharp and constant. Since the drugs contain the mixed chemicals, they are described with certain range of melting point.

Procedure for determine melting point

A limited quantity of powder was arranged into a fusion tube. That tube was arranged in the melting point determining apparatus (Chemline) include castor oil. The temperature of the castor oil was gradual increased automatically and read the temperature at which powder begun to melt and the temperature when all the powder gets melted.

RESULT OF MELTING POINT

Table 4: Melting point of Acyclovir.

S. No.	Melting Point of Acyclovir	Average Melting Point of Acyclovir
1.	256-258^{o}C	256-258^{o}C
2.	256-258^{o}C	
3.	257-259^{o}C	

D) Determination of pH (1 w/v solution in water)

Procedure

About 100mg of the active drug was taken and dissolved in 10ml of distilled water with sonication and refine. The pH of the filtrate was checked with standard glass electrode.

Result of Determination of pH (1% w/v solution in water)

Table 5: pH of the Acyclovir.

S. No.	pH of the solution	Average pH of the solution
1.	7.2	
2.	7.1	7.2
3.	7.2	

E) Partition coefficient: It is a measurement of a drug's lipophilicity and an indication of its ability to cross cell membrane is the oil/water partition coefficient in order such as octanol/water and chloroform/water. The partition coefficient is defined as the ratio of un-ionized drug distributed between the organic and aqueous phases at equilibrium. It does provide a mean of characterizing the lipophilic/hydrophilic nature of the drug.

Procedure

Taken well cleaned and dry separating funnel, then transversed the octanol/water system (50:50 20ml) as satisfactory quantity in distribution funnel and added the 10mg drug in it. Shaked the funnel continuously until the drug was delivered in both phases. Then placed the funnel on stand for settle both phases. After that taken both phases in beaker distribute and determined the drug amount present in both phases.

Result of Partition coefficient

Partition coefficient (log P) value of Acyclovir was found to be **-1.59.**

F) Identification Test

FTIR Spectroscopy

Infra- red spectrum is an valuable record which gives sufficient report about the structure of a mixture. This technique give a spectrum containing a large number of absorption band from which a wealth of report can be derived about the structure of an organic compound. The range from 0.8 µ to 2.5 µ is known as Near Infra-red and that from 15 µ to 200 µ is known as Far infra-red range.

Identification of Acyclovir was done by FTIR Spectroscopy with respect to marks compound. Acyclovir was obtained as White or almost white crystalline powder. It was identified from the result of IR spectrum as per specification.

Sample of pure Acyclovir

The IR spectrum of sample drug shows the peak principles which are characteristics of the drug and the graph were display in figure.

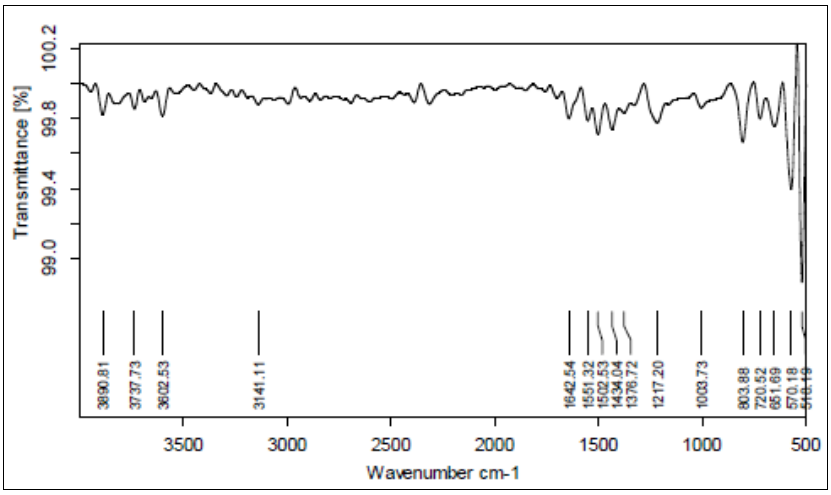

Figure 2: FT-IR Spectrum of Pure Drug (Acyclovir).

G) Loss on drying: The moisture in a solid can be convey on a wet weight or dry wet basis. On a wet weight basis, the water content of a material is determined as a percentage of the weight of the weight solid. The word loss on drying is an expression of moisture content on a wet weight basis.

Procedure

Loss on drying is directly determined by IR moisture balance. Firstly calibrated the apparatus by knob then taken 5 gm sample (powder) and set the temp at 100°C to 105°C for 15 minutes and constant reading set the knob and check % moisture.

RESULT OF LOSS ON DRYING

Table 6: Loss of drying of drug sample.

S. No.	Initial weight	Final weight after 15 minutes	% loss of drying	Avg. % loss of drying
1.	5gm	4.92 gm	1.67 %	
2.	5gm	4.91 gm	1.82 %	1.672 %
3.	5gm	4.92 gm	1.67 %	

H) Moisture content determination

Principle: The titrimetric determination of water is located upon the quantitative reaction of water with an anhydrous solution of sulphur dioxide and iodine in the existance of a buffer that perfrom with hydrogen ions.

In the original titrimetric solution, are known as Karl Fisher Reagents, the sulfur dioxide and iodine was dissolved in pyridine and methanol. The test specimen may be titrated with the reagent directly, or the analysis may be carried out by a residual titration method. The stoichiometry of the reaction is not exact, and the reproducibility of a determination depends upon such factors as the relative concentration of the reagent component, the nature of the inert solvent worn to dissolve the test specimen, and the method used in the particular resolve. Therefore, an empirically standardized technique is used in order to achieve the desired accuracy. Precision in the method is governed widely by the extent to which atmospheric moisture is excluded from the organization. The aquous solution is mostly carried out with the help of anhydrous methanol as the main solvent for the test specimen. however new applicable solvents may be worn for special or unusual test specimens. (Note: Now-a-days pyridine free KF reagents are preparing in which pyridine is replaced by the imidazole, because pyridine has carcinogenic effects).

Procedure

Karl Fischer volumetry is worn for samples with large water content, *i.e.* 1-100 mg per sample. An iodine-containing solution serves as titrating agent. The water content of the sample is calculated using titration volume and titer of the titrating agent. One-component reagents conveniently contain all reactants (iodine, sulfur dioxide and a base) dissolved in a suitable alcohol in one solution, whereas two-component reagents contain all necessary reactants separated in two different solutions to enhance the velocity of the Karl Fischer reaction and the titer stability of the titrating agent.

Karl Fischer coulometry is a micro-method and is particularly suitable for samples with below water content, from 10 µg up to 10 mg. Here, the required iodine is generated electrochemically in the titration vessel by anodic oxidation from iodide contained in the coulometric reagents. The amount of consumed electric charge is used to calculate the consumption of iodine and therefore the amount of water in the sample.

Result of Moisture content determination

Table 7: Moisture content determination.

S. No.	Drug	KF Factor	Amount of KF Reagent consumed	Moisture content
1	Acyclovir	0.565	0.13ml	0.07345

I) Determination of λ_{max} of Acyclovir

The λ_{max} of Acyclovir was determined by running the spectrum of drug solution in double beam ultraviolet spectrophotometer.

Procedure

Correctly measure 10mg of drug was diluted in 10ml of 7.2 pH buffer diluted in 10ml of volumetric flask. The resulted diluted 1000µg/ml and from this sol^{n} 1 ml pipette out and move into 10 ml volumetric flask and volume make up with 7.2 pH buffer sol^{n} prepare suitable dilution to make it to a concentration range of 5-25 µg/ml. The spectrum of this diluted was run in 200-400nm range in U.V. spectrophotometer (Labindia-3000+). The spectrum peak graph of absorbance of Acyclovir versus wave length was presented in figure.

Results of Determination of λ_{max} of Acyclovir

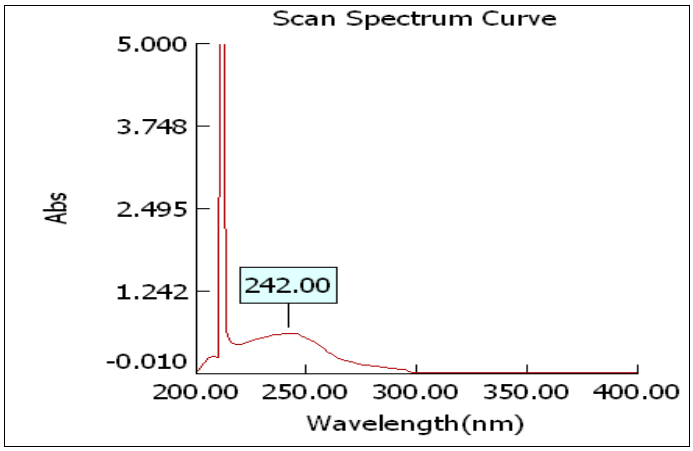

Figure 3: Wavelength maxima of Acyclovir in phosphate buffer pH 7.2.

Calibration curve of Acyclovir at λ_{max} 242nm

Table 8: Calibration curve of Acyclovir.

S. No.	Conc. (µg/ml)	Absorbance
1	5	0.278
2	10	0.528
3	15	0.748
4	20	0.984
5	25	1.196

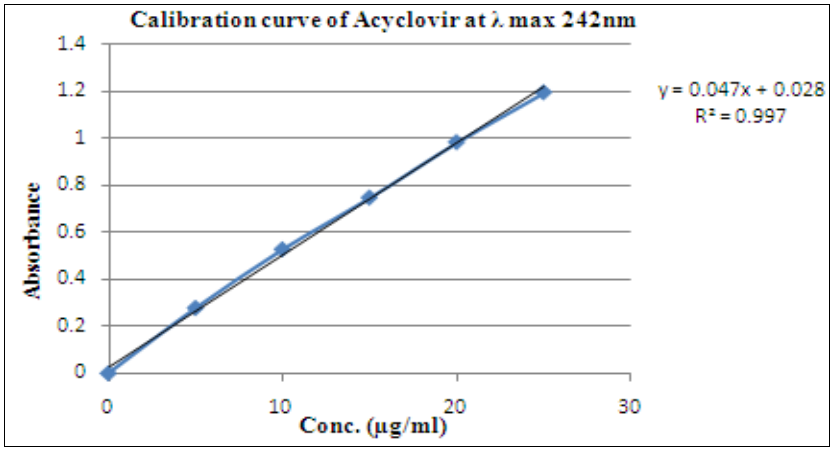

Figure 4: Calibration curve of Acyclovir in phosphate buffer pH 7.2 at 242nm.

The linear regression analysis was done on Absorbance data points. The results are as follow for standard curve

Slope = 0.047

The intercept = 0.028

The correlation coefficient (r^2) = 0.997

Compatibility studies of drug and excipients

In the compatibility experiment program, blends of drug and excipients are processed by triturating the drug with Individual excipients.

Procedure: Taken 50 mg accurately weigh of Acyclovir dry powder and 50 mg of excipients and mix the blend of drug and excipients and binary/tertiary blends of extract and excipients were arranged and transferred to inert glass vials. The mouths of the vials were covered with rubber closures followed by the aluminum seal caps. Binary/tertiary blends of extract and excipients, Acyclovir neat and excipients were stored at 4˚C (refrigerator) as control and at 40˚C/75%RH for accelerated stability studies for 4 weeks. The visual observations (color, flow, & sticking) were recorded for initial and at the edge of the first, second, third and fourth week.

Compatibility studies of active drug and excipients

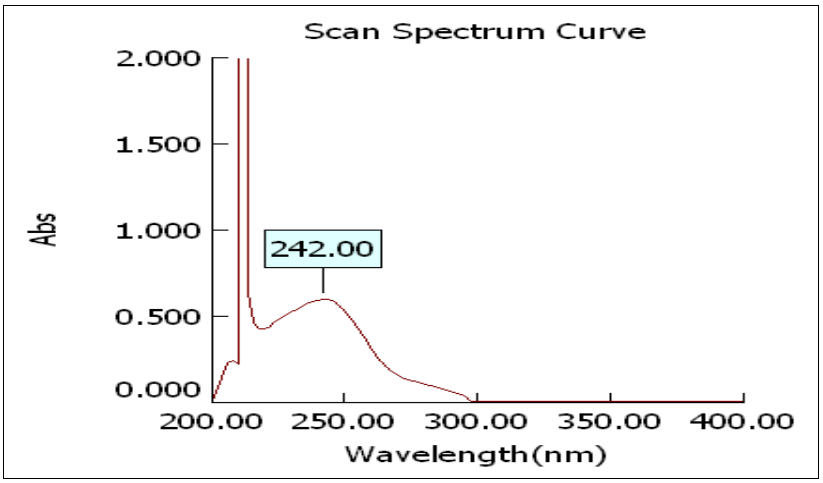

Figure 5: U.V. Estimation of Pure Acyclovir.

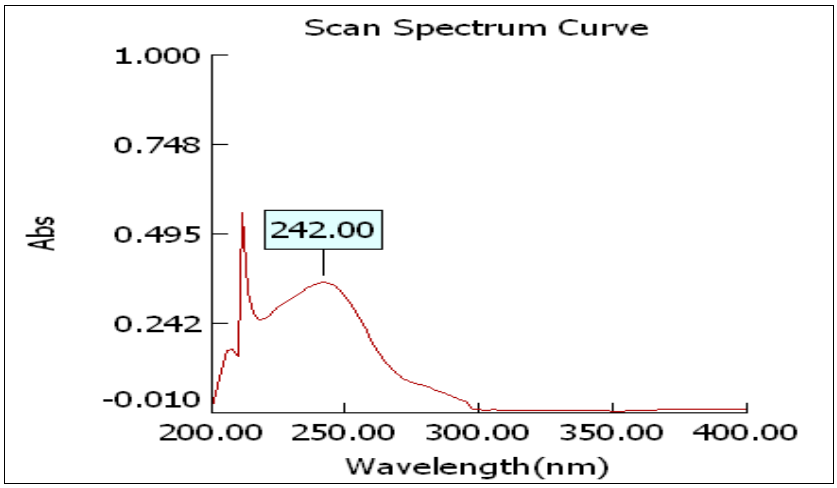

Figure 6: U.V. Estimation of Pure Acyclovir + All Excipients.

Formulation, development acyclovir loaded Transfersomes

1 Preparation of Acyclovir loaded Transfersomes

Soya PC (1% w/v) was dissolved in ethanol (25-45% v/v) and heated up to 30 ± 1°C in a water bath in a closed vessel. Distilled water or drug solution in distilled water (1% w/v solution), which is previously heated up to 30 ± 1°C, was added slowly in a fine stream to the above ethanolic lipid solution with continuous mixing using a magnetic stirrer at 900 rpm. Mixing was continued for another 5 minutes and finally, the vesicular dispersions resulted was left to cool at room temperature (25 ± 1°C) for 45 minutes.

2 Preparation of Gel Base

Carbopol 934 (1%w/v) was accurately weighed and dispersed into double distilled water (80ml) in a beaker. This solution was stirred continuously at 800 rpm for 1 hour and then

10ml of propylene glycol was added to this solution. Volume of gel was adjusted to 100 ml and then sonicated for 10 min on bath sonicator to remove air bubbles. Final pH of the gel base was adjusted to 6.8. Transferosomal preparation corresponding to 2% w/w of Acyclovir was incorporated into the gel base to get the desired concentration of drug in gel base.

Table 9: *In vitro* drug release study of prepared gel formulation.

S. No.	Time (hr)	% Cumulative Drug Release
1	0.5	13.56±0.45
2	1	27.56±0.65
3	2	35.65±0.23
4	4	45.65±0.32
5	6	65.58±0.41
6	8	75.65±0.98
7	12	89.56±0.23

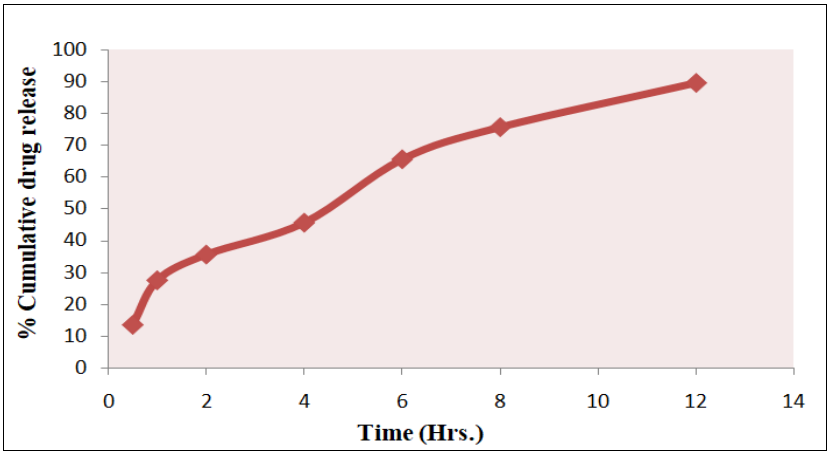

Figure 7: *In vitro* drug release of gel based transferosomal gel.

Optimization of Transfersomes

1 Optimization of lipid

In the transferosomal formulation, the ratio of lipid was optimized by taking their different ratio such as 0.5, 1.0, 1.5 and 2.0% ratio and all other parameters were kept remain constant. The prepared formulations were optimized on the basis of average vesicle size and % entrapment efficiency.

Table 10: Optimization of lipid concentration.

Formulation code	Soya PC (% w/v)	Ethanol	Drug (% w/v)	Average vesicle size (nm)	% entrapment efficiency
F1	0.5	10	1.0	352.23	76.65
F2	1.0	10	1.0	269.85	75.12
F3	1.5	10	1.0	256.23	65.65
F4	2.0	10	1.0	245.32	63.12

Table 10: Optimization of ethanol concentration.

Formulation code	Soya PC (% w/v)	Ethanol	Drug (% w/v)	Average vesicle size (nm)	% entrapment efficiency
F5	1.0	5	1.0	265.56	62.32
F6	1.0	10	1.0	220.32	75.45
F7	1.0	15	1.0	275.65	55.56
F8	1.0	20	1.0	232.12	63.32

2 Optimization of drug concentration

Drug concentration was optimized by taking different concentration of drug and prepared their formulation and all other parameter such as Soya PC, stirrer time kept remains constant. The formulation optimized on the basis of entrapment efficiency and average vesicle size.

Table 11: Optimization of drug concentration.

Formulation code	Soya PC (% w/v)	Drug (% w/v)	Ethanol (ml)	Average vesicle size (nm)	% Entrapment efficiency
F9	**1.0**	**1.0**	**10**	**245.52**	**79.98**
F10	1.0	1.5	10	269.25	65.52
F11	1.0	2.0	10	298.65	62.12

3 Optimization of stirrer time

Stirrer time was optimized by Stirrer the formulation for different time i.e 5, 10 and 15 min. The optimization was done on the basis of average vesicle size, and % Entrapment efficiency.

Table 12: Optimization of Stirrer time.

Formulation code	Soya PC: (% w/v)	Drug (% w/v)	Stirrer time (min)	Average vesicle size (nm)	% Entrapment efficiency
F12	1.0	1.0	5	152.23±0.25	82.23±0.45
F13	1.0	1.0	10	145.65±1.25	65.45±1.15
F14	1.0	1.0	15	130.25±1.30	45.65±1.20

Average Vesicle Size and Zeta Potential

Prepared formulations of Transfersomes were optimized on basis of vesicle size, shape, surface charge and entrapment efficiency. Vesicle size of Transfersomes were examined under trinocular microscopic (magnification 400X) and also determined by light scattering method (Malvern Zetasizer, ZEM 5002, and UK) and found that average vesicle size of optimized formulation F-12 was 152.23 nm. Zeta potential was -25.65 (It was observed that the vesicles size of Transfersomes was increase with increasing the concentration of phosphotidylcoline and similarly vesicle size. There was no significant difference in average

vesicle size was observed with increasing the drug concentration. But in increasing the stirrer time the size vesicle was decrease from 152.23 to 130.25 after 15 min of steering.

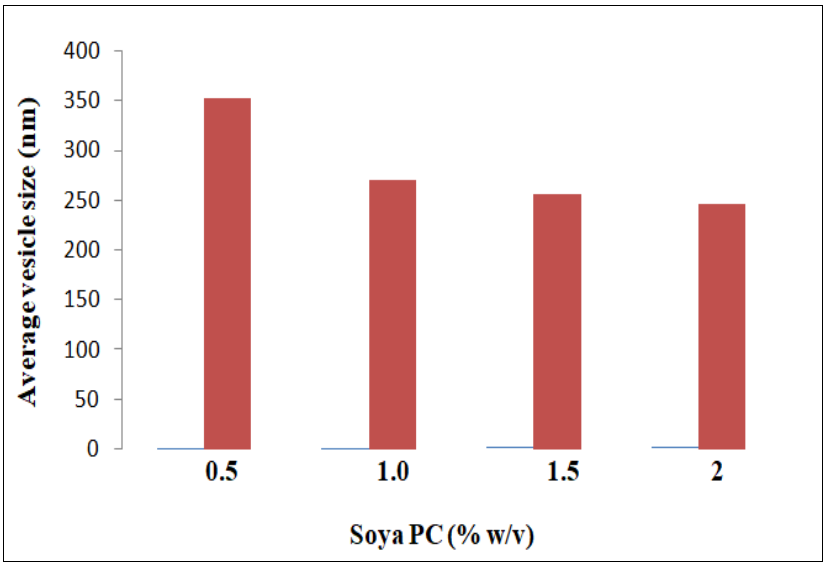

Figure 8: Effect of different ratio and concentration of soya PC on Vesicle size.

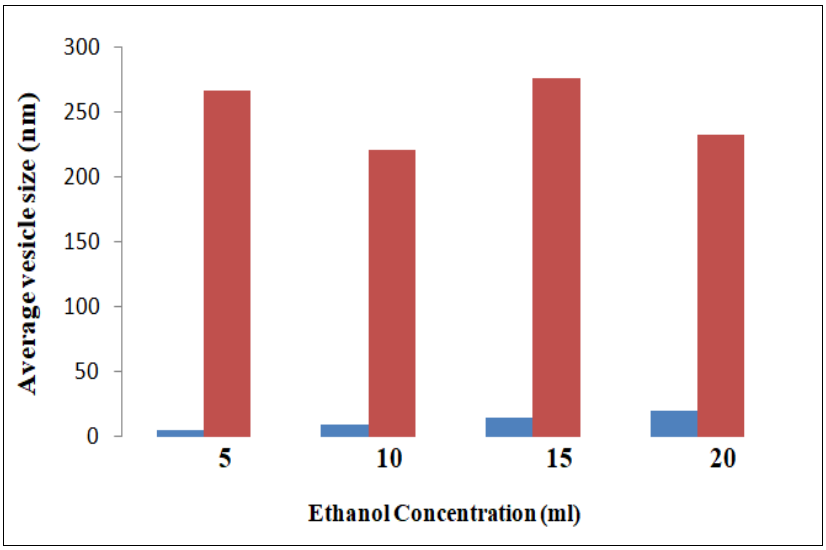

Figure 9: Effect of ethanol concentration on vesicle size.

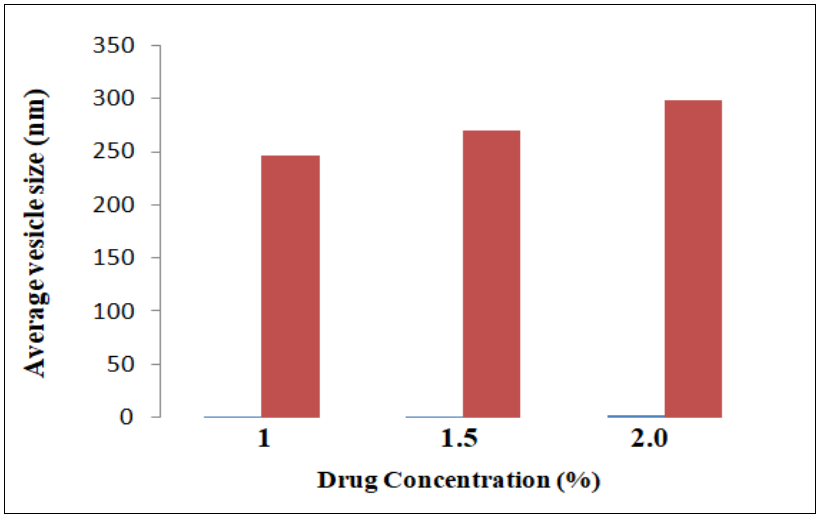

Figure 10: Effect of drug concentration on vesicle size.

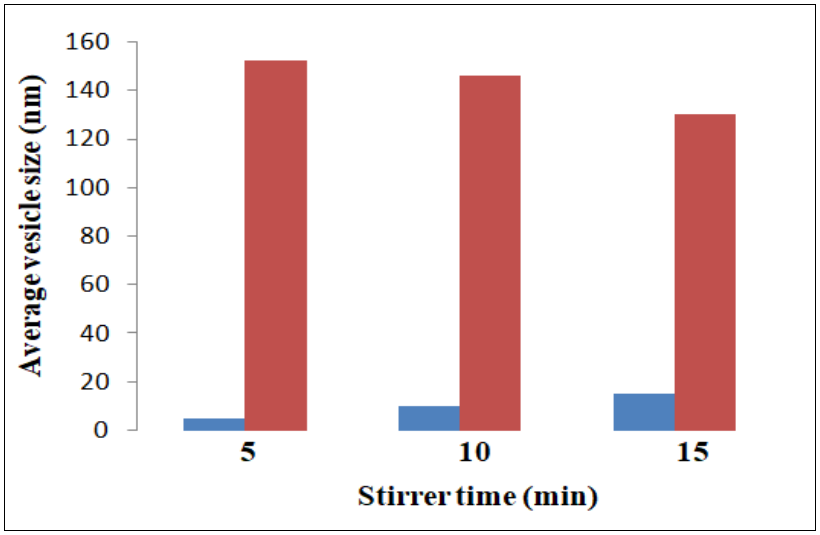

Figure 11: Effect of Stirrer time on vesicle size.

7.3.2 % Entrapment efficiency

% Entrapment efficiency of optimized transferosomal formulation (F-12) was found 78.03±1.45%. It was observed that the percent drug entrapment was decrease with increasing the concentration of ethanol and on increasing the time of starring. It is due to the leaching out the drug from vesicles on increasing the mechanical force by stirrer and size reduction of size Transfersomes on increasing the concentration of ethanol. It was clearly shown when formulation was stirrer for 5, 10 15 min then the % EE was 82.23±0.45, 65.45±1.15 and 45.65±1.20. The 5 min is selected as optimized time for stirrer because it provided the required size of vesicle 152.23±0.25nm and good % EE i.e 82.23±0.45. The F-12 formulation was selected as optimized formulation.

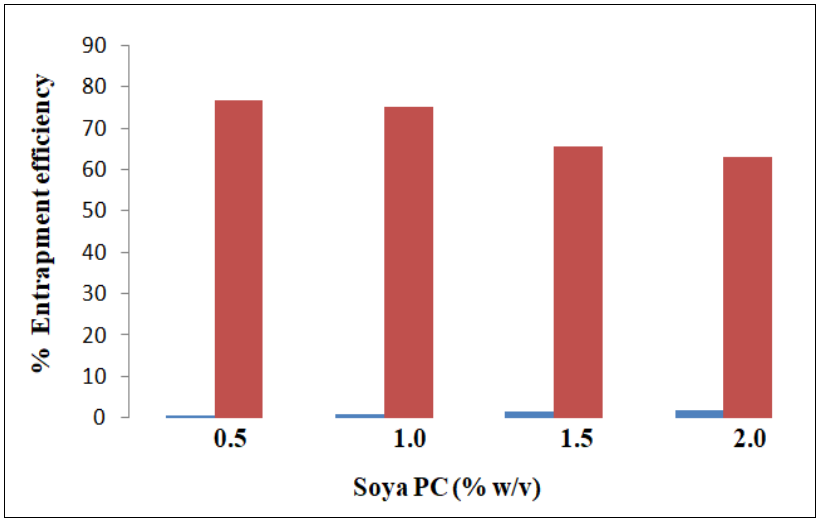

Figure 12: Effect of different ratio and concentration of soya PC on %EE.

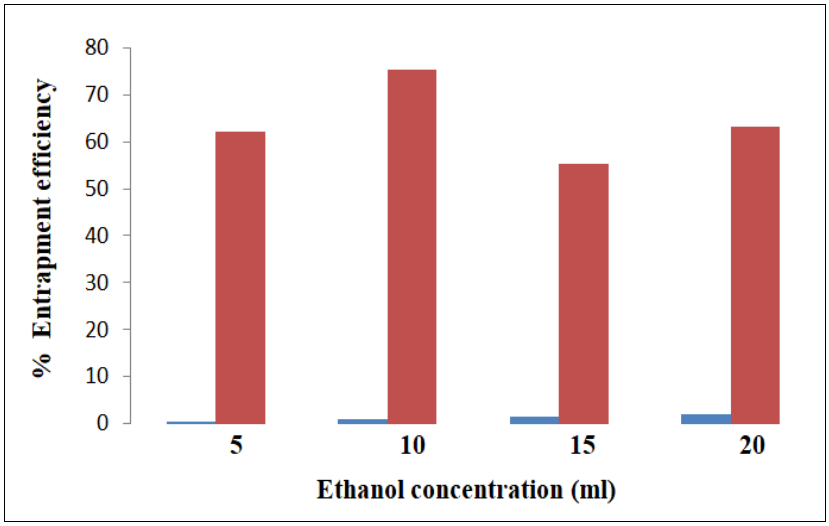

Figure 13: Effect of ethanol concentration of drug on % EE.

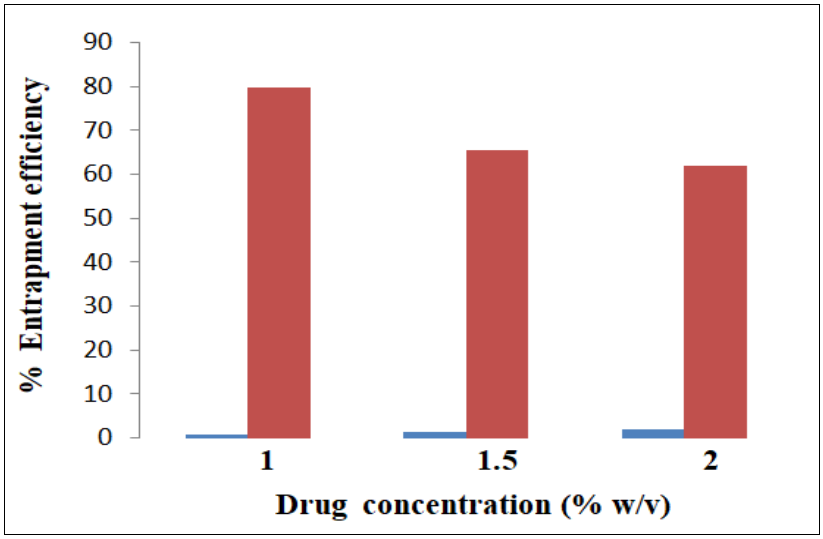

Figure 14: Effect of drug concentration of drug on % EE.

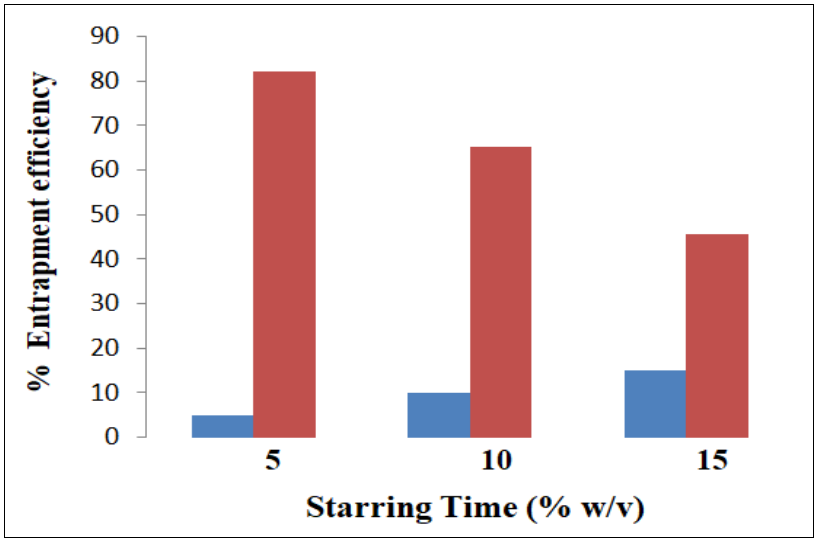

Figure 15: Effect of starring time on % EE.

- The preliminary study showed that acyclovir is white, crystalline, odorless powder. It is Freely soluble in Ethanol, Methanol and 0.1 N HCL soluble in chloroform, slightly soluble in water. The melting point was in the range of 256-258^{o}C which is in compliance with the standard value of 256 $^{\circ}$C as per Indian Pharmacopoeia.
- From the FTIR data of the physical mixture it is clear that functionalities of drug have remained unchanged including intensities of the peak. This suggests that during the process drug and cholesterol has not reacted with the drug to give rise to reactant products. So there is no interaction between them which is in favor to proceed for formulation of vesicular drug delivery system. The U.V study shows that the drug and cholesterol are compatible with each other. Preformulation studies reported that the formulation of nanosponges of acyclovir can be prepared with appropriate methods.
- Transfersomes are advantageous as phospholipids vesicles for transdermal drug delivery. Because of their self-optimized and ultra flexible membrane properties, they are able to deliver the drug reproducibly either into or through the skin, depending on the choice of administration or application, with high efficiency. The vesicular transfersomes are more elastic than the standard Transfersomes and thus well suited for the skin penetration. Transfersomes overcome the skin penetration difficulty by squeezing themselves along the intracellular sealing lipid of the stratum corneum
- Skin permeation profile of acyclovir through transfersomes formulations was observed in the present study. The investigations revealed an enhanced transdermal delivery of acyclovir. Hence, it could be concluded that transfersomes may be suitable carrier for transdermal delivery of acyclovir.

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